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Journal of the American Association of Clinical Chemists

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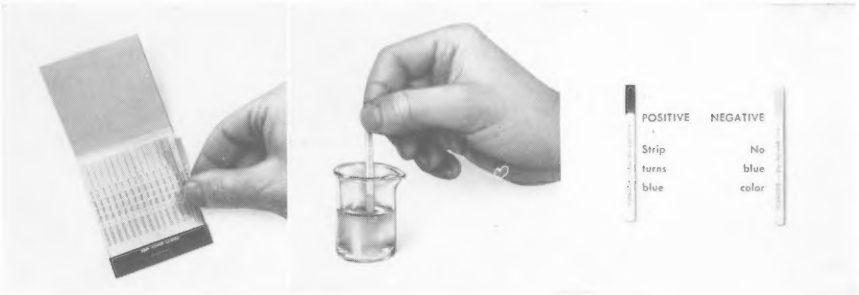

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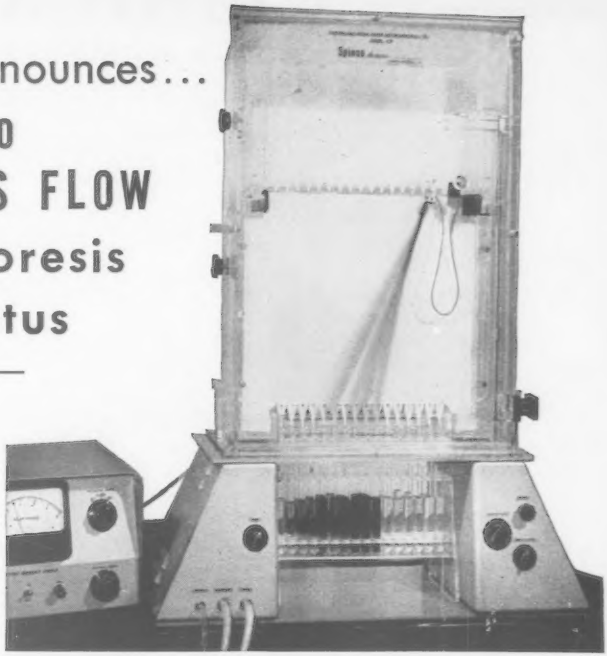

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International Congress on Clinical Chemistry Sept. 9-14, 1956 New York, N. Y.

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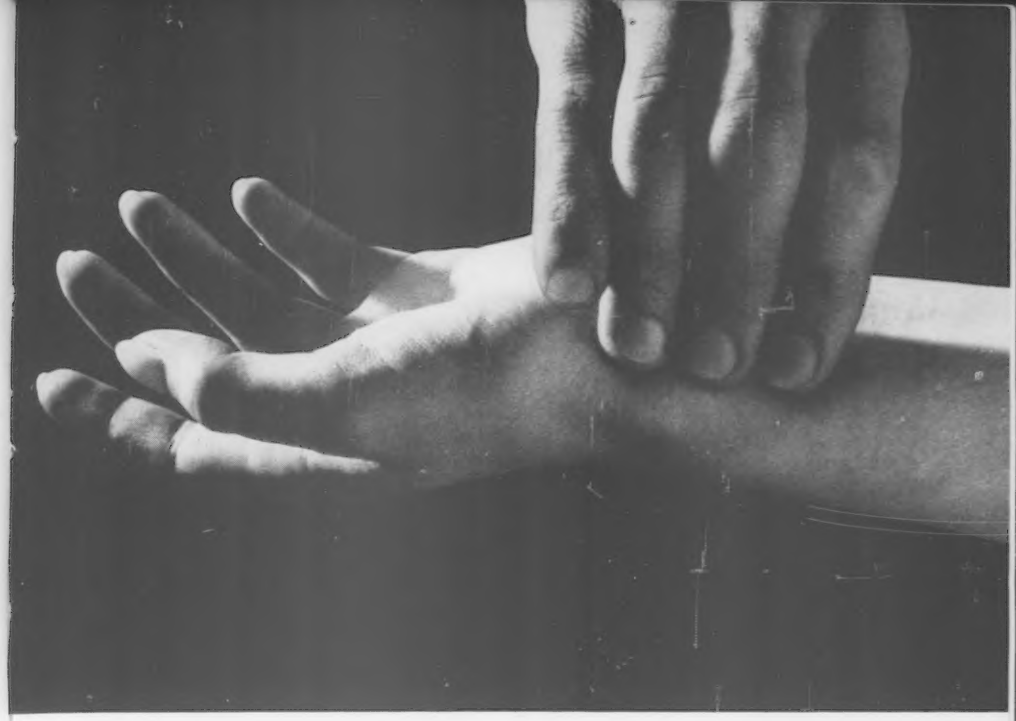

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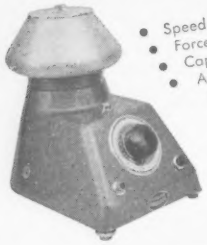

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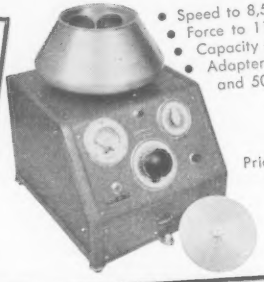

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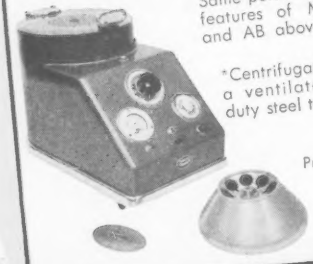

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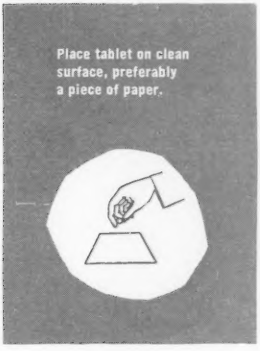

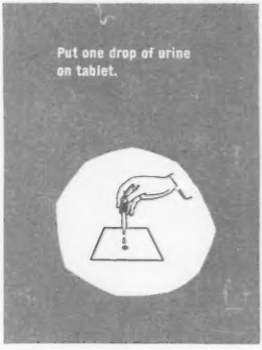

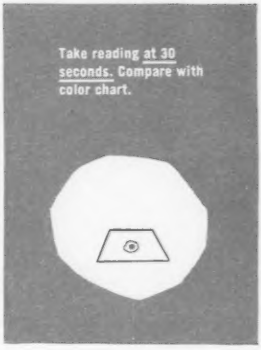

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*Nash, J.; Lister, J., and Vobes, D. H.: Lancet *1*:801 (April 17) 1954.

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CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

VOLUME 2 AUGUST 1956 NUMBER 4

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INTERNATIONAL CONGRESS ON CLINICAL CHEMISTRY

The Barbizon-Plaza Hotel, New York, September 9-14, 1956

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ABSTRACTS

from the

International Congress on Clinical Chemistry

New York, N. Y., September 9–14, 1956

SCIENTIFIC PAPERS

SESSION 1. Symposium on Blood Electrolytes: I

A

Appreciation of the contribution to clinical chemistry by the late Dr. J. P. Peters. D. D. Van Slyke (*Brookhaven National Laboratory, Upton, N. Y.*)

B

Blood electrolytes. C. P. Stewart (*Dept. of Clinical Chemistry, Royal Infirmary, University of Edinburgh, Scotland*)

The available time permits only brief reviews of a few selected topics. Apart from some general considerations of the value of clinical chemistry and of blood analysis in the investigation of disturbed electrolyte metabolism, it is proposed to discuss some recent work on: (1) the plasma electrolytes in disturbances of the acid-base balance; (2) the electrolyte pattern following surgical operation and its relation to adrenocortical activity; (3) the diagnosis of sodium and potassium depletion and the control of treatment; and (4) the diagnosis and control of conditions involving renal loss of potassium, including "primary aldosteronism."

C

Blood electrolytes. R. Margaria (*Laboratorio di Fisiologia, Universita di Milano, Milan, Italy*)

A few topics that have aroused a particular interest in recent times are discussed:

The problems of drinking sea water in cases of prolonged periods of survival at sea has met much controversy, particularly in relation to an experiment by Bombard (1953). Laboratory experiments on the concentrating capacity of human urine would show the incapacity of the kidney to eliminate electrolytes at a higher concentration than in sea water, and therefore ingestion of sea water would not be recommendable.

The practice of hibernation in surgery is finding more and more application. Disturbance of the acid-base equilibrium in this condition is due to various factors such as lower activity of the respiratory center, higher solubility of CO_2 at lower temperature, lower dissociation of H_2CO_3 and of proteins, production of organic acids by the cooling body. These functions are described in order to limit as much as possible deviations from the normal equilibrium value with compensating mechanisms.

Respiratory disturbance involves a disturbance of the acid-base equilibrium and this in turn a displacement of the equilibrium between different electrolytes in blood. Base reabsorption in the kidneys depends on the concentration of the

single cations in blood, on the *p*H and P_{CO_2}, these last being related to the reabsorption of bicarbonate. Disturbances in these mechanisms may give cause to clinical symptomatology and functional disorders in respiratory patients.

D

The role of magnesium in the body fluids. J. R. Elkinton (*Chemistry Section, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa.*) (Abstract to follow)

SESSION 2. Serum Proteins in Hepatic Disease

1

Cupric ferrocyanide as a measure of "serum protein imbalance" and hepatoparenchymal dysfunction. E. A. Napier, Jr. (*Veterans Administration Hospital, Leech Farm Road, Pittsburgh 6, Pa.*)

Cupric ferrocyanide reagent buffered with barbital at *p*H 7.55 and ionic strength 0.01 has been found to be readily adaptable to the routine estimation of "serum protein imbalance" and liver anomalies arising from hepatoparenchymal involvement.

Upon the addition of serum to the buffered cupric ferrocyanide reagent, the amount of reagent precipitated after centrifugation may be calculated by analyses of an aliquot of the supernatant to note the amount of "colloidal copper" present. Through the use of copper specific colorimetric complexing reagents such as 2,9-dimethyl-1,10-phenanthroline hemihydrate, an accurate routine measure of precipitation of the electronegative colloid by serum proteins may be undertaken. "Serum protein imbalance" may thus be represented as "per cent colloidal copper precipitated."

The method has been found to be favorable in delineating hepatoparenchymal dysfunction cases (as cirrhotics) from a random group of nonliver medical cases and normals, and yields a high correlation with the thymol turbidity test. Ease in standardization, quantitation, and the stability of the cupric ferrocyanide reagent, however, offer advantages over classical nephelometric methods such as the thymol turbidity test.

2

On the quantitative cephalin-cholesterol flocculation test for liver function. A. C. Kibrick, A. S. Gargano, and S. J. Skupp (*Chemistry Section, Department of Clinical Laboratories, New York Veterans Administration Hospital, New York, N. Y.*)

Additional evidence is presented for the usefulness of the quantitative method for the determination of cephalin-cholesterol flocculation with photometric reading of the absorption in the supernatant fluid obtained by centrifugation. Although results calculated in per cent of the absorption in the blank tubes differ somewhat for different photoelectric instruments and for different-sized cuvets, and hence are not capable of identical interpretation, a value of 40 per cent may be assumed to be quite close to the upper limit of normal for all instruments and for all cuvets. Values of cephalin-cholesterol flocculation calculated from the cholesterol contents of the precipitates as in the Saifer method and from the cholesterol contents of the supernatant fluids roughly approximate the results obtained by

photometric reading of turbidity. However, discrepancies are quite apparent. There is much more overlapping of the results obtained from the cholesterol values in normals or in patients without liver disease and those in patients with liver damage. This is undoubtedly due to the considerable variation which we have found in the cholesterol content of commercial antigen and to the variation in the amount of cholesterol present in the serum samples. An attempt was made to adjust the reaction tubes to a constant content of cholesterol. However, this was academic, since the proposed photometric method is certainly more convenient even without adjustment for constant cholesterol, and since this method has been found reliable in many hundreds of tests.

3

The role of the basic fraction of γ-globulin in the flocculation tests. E. C. Franklin and H. G. Kunkel. (*The Rockefeller Institute for Medical Research, New York, N. Y.*)

The thymol turbidity, zinc turbidity, and cephalin flocculation tests reflect in part changes in γ-globulin. Some observers have noted that γ-globulin from hepatitis serum reacts more strongly with thymol reagent than normal γ-globulin. Therefore, an attempt was made to determine whether all fractions of γ-globulin react similarly with the flocculating reagents and whether qualitative changes in the γ-globulin might explain abnormalities in the flocculation tests.

Cohn Fraction II-γ-globulin, and γ-globulin from normal and pathological sera were separated by starch zone electrophoresis into 3–10 subfractions with different mean mobilities. Equal aliquots of each fraction were used in the flocculation tests. A small amount of lipid was added in the thymol turbidity test. The basic fraction of the γ-globulin reacted most vigorously in the thymol turbidity and cephalin flocculation tests, while the more acidic γ-globulin was inactive. In the zinc turbidity test the fraction of intermediate mobility was most active.

To further evaluate the role of the charge of the γ-globulin, proteins with different isoelectric points were tested. Basic proteins such as lysozyme, ribonuclease, and trypsin were very active in the thymol turbidity and cephalin flocculation tests, while acidic proteins such as β-lactoglobin, ovalbumin, hemoglobin, and serum albumin and α- and β-globulin reacted weakly or not at all.

Groups of sera were separated simultaneously by starch zone electrophoresis and the distribution of the γ-globulin was determined. An increase in the basic portion of the γ-globulin was usually found in sera with positive thymol turbidity and cephalin flocculation tests. Certain exceptions were encountered which may be due to alterations in the serum lipoproteins or albumin.

4

In vitro globulin fractional analysis as an aid in the differentiation of medical from surgical jaundice. E. M. Greenspan. (*Department of Medicine, The Mount Sinai Hospital, New York, N. Y.*)

Quantitative distribution of serum α-, β-, and γ-globulin may be estimated by a battery of four simple procedures measuring α_1-mucoprotein (M), acid-precipitable globulin (APG) turbidity, zinc sulfate (ZS) turbidity, and the protein-bound polysaccharide (PTP).

A falling serum α_1-mucoprotein (M) level with a rising γ-globulin, i.e. zinc sulfate (ZS) turbidity, represented strong confirmatory evidence in the jaundiced patient for the diagnosis of infectious or homologous serum hepatitis, or the presence of portal cirrhosis. An increased α_2- plus β-globulin content, as measured by the acid-precipitable globulin (APG) turbidity level, was a frequent finding in acute or chronic biliary obstructive disease. When biliary obstruction was due to an active inflammatory process or an extensive neoplasm the M level usually increased and was accompanied by a high APG turbidity, while the ZS turbidity usually remained normal or was decreased. This contrasts with the characteristically reduced M levels and rising ZS levels associated with hepatocellular jaundice. The profile of reduced M and APG values with high ZS and normal PTP was almost invariably associated with severe advanced hepatocellular insufficiency due to portal or post-necrotic cirrhosis. The reciprocal relationship of the APG-ZS turbidity ratio in medical and surgical jaundice will be demonstrated. Fractional analysis of the globulin profile by these methods offered practical advantages over electrophoresis, and compared favorably with older procedures frequently employed in a battery of liver diagnostic aids.

5

Studies on the zinc sulfate turbidity test. A. Hainline, Jr., T. E. Wilson, C. H. Brown and Lena Lewis. (*The Cleveland Clinic Foundation and the Frank E. Bunts Educational Institute, Cleveland, Ohio*)

The zinc sulfate turbidity test was introduced as a technic for estimating the level of γ-globulin in serum. Subsequent study has indicated that the zinc sulfate turbidity is affected by serum albumin levels as well as γ-globulin levels. Results of zinc sulfate turbidity tests were compared with Tiselius electrophoretic protein patterns of the serum from 174 patients. Comparison of the zinc sulfate turbidity with globulin levels gave the following correlation coefficients: with γ-globulin 0.59, with albumin–γ-globulin ratio 0.63, with α- or β-globulin no significant correlation. The zinc sulfate turbidity was compared with total serum globulin levels in 273 patients; the correlation coefficient was 0.39. A total of 1187 individual clinical diagnoses were reviewed and compared with zinc sulfate turbidities. Normal values ranged from 4 to 12 units. Elevated tests were found in patients with the following diagnoses: functional disorders, 9 of 392; noninfectious, nonhepatic organic disease, 57 of 494; cirrhosis, 118 of 179; hepatitis, 20 of 54; obstructive jaundice, 4 of 42. Statistical comparison between normal and abnormal groups indicates that the zinc sulfate turbidity is a valuable test in diagnosing hepatobiliary disease. The zinc sulfate turbidity shows abnormal levels more frequently in cirrhosis than do other turbidity or flocculation tests. When normal in jaundice of long duration, it is a nearly specific indication of a surgical cause of the jaundice in the absence of hemolytic anemia.

6

Carbon dioxide and the reaction between zinc ions and serum proteins. J. G. Reinhold and V. Yonan (*William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa.*)

It has been observed in this laboratory that repetition of zinc turbidity measurements in the afternoon consistently gave lower readings than those originally

obtained in the morning. A similar though more marked change occurred on standing overnight. On the other hand, collection of specimens and subsequent processing under anaerobic conditions prevented such changes. The most plausible explanation was that carbon dioxide was the variable responsible for these changes. Experiments have been designed to provide evidence of a role of CO_2 in the zinc sulfate turbidity test. Removal of free CO_2 from sera by exposure to a vacuum markedly lowered the zinc turbidity reading. Exposure of the serum to a gas phase rich in CO_2 increased the readings. The differences were not explained by changes in the *p*H of the final reaction mixture. Because of this strong evidence that CO_2 is involved in the reaction between zinc and proteins, consideration should be given this factor in performing this test. These findings suggest also that the reaction of zinc with protein may involve linkage through carbamyl groups.

7

Serum iron, iron-binding capacity, and C-reactive protein in liver disease. E. E. Mandel (*The Chicago Medical School and Mt. Sinai Hospital, Chicago, Ill.*)

Serum iron was measured by means of a single stable reagent containing 2:2′:2″-tripyridine (0.004%) and hydroxylamine HCl (0.05%) in 0.5M acetate buffer. The total iron-binding capacity (TIBC) was determined by applying this method to another milliliter of serum, previously supersaturated with a ferric compound and treated with resin IRA-410 which removes all excess iron (checked with Fe^{59}). C-reactive protein (CRP) was estimated with commercial antiserum.

The serum iron was elevated (above 150 μg. per 100 ml.) in 22 of 44 cases of acute (viral or toxic) hepatitis, 4 of 38 patients with biliary obstruction and 7 of 37 cirrhotics. It was decreased (below 50 μg.) in 3, 8, and 11, respectively. TIBC was increased (above 400 μg. per 100 ml.) in 15 of 29 cases of hepatitis, but decreased (below 300 μg.) in 17 of 21 patients with cirrhosis.

Both iron and TIBC elevations in hepatitis usually coincided with, or appeared shortly after, the peak of jaundice. At the same time, the C-reactive protein tended to be negative (19 of 24 cases), even if positive at the outset of the disease; it was, as a rule, positive in cirrhosis and in obstructive jaundice. The characteristic laboratory pattern of acute viral hepatitis includes a high serum iron and TIBC and a negative CRP; the opposite is true in cirrhosis and in advanced biliary obstruction (neoplasm), while early obstruction tends to be associated with normal iron and TIBC values.

8

Chelating agents and iron metabolism. J. V. Princiotto and M. Rubin (*Department of Physiology, Schools of Medicine and Dentistry, and Department of Chemistry, Georgetown University, Washington, D. C.*)

It is known that the absorption, transport, and distribution of iron occurs through the mediation of several iron-combining compounds. A rational approach to the problems of iron therapy and pathology therefore requires some knowledge of the quantitative aspects of the interrelation of synthetic and naturally occurring iron compounds. For this reason an in vitro evaluation of the iron-binding strengths of a series of synthetic chelating agents were compared with the iron-

binding strength of siderophilin. It was concluded that ethylenediamine-tetraacetic acid (I) and β-hydroxyethyl-ethylenediamine-triacetic acid (II) were close to the strength of the transporting protein, siderophilin, in iron-binding ability. These results suggested that one could influence the ultimate distribution of iron in the body by the administration of synthetic compounds of appropriate iron-binding strength.

Intravenous iron given as the chelates of I and II resulted in the urinary excretion of approximately 70 per cent of the injected iron. Upon I.M. administration the ethyl ester of II was converted to the free chelate in vivo and a three- to fourfold increase in urinary iron excretion resulted. The ability of the body to excrete iron when that iron can be converted to a form amenable to excretion as shown by these results has offered a possible chemotherapeutic approach for iron storage disease.

In our ferrotherapy studies the oral administration of iron as the chelates of I and II and as iron sulfate clearly indicated that despite the iron binding gradient all three compounds were equally efficacious in eliciting a hemoglobin rise. The distinctly graded hemoglobin response following the I.P. administration of a series of iron chelates was in the same direction as their relative iron binding strengths. The I.V. and I.M. administrations of Fe I and II in dosage levels adjusted in accordance with our urinary iron excretion data showed a prompt hemoglobin response.

These in vivo results suggest a new approach to I.M. ferrotherapy.

SESSION 3. Electrolytes

9

Turbidimetric Estimation of Potassium in Biological Fluids with Tetraphenylboron. M. H. Power and Catherine Ryan (*Section of Biochemistry, Mayo Clinic and Mayo Foundation, Rochester, Minn.*)

Recent applications of tetraphenylboron as a reagent for potassium in inorganic analysis have led to examination of the possible uses of this new compound in biochemical analysis. Preliminary trial of a simple procedure involving development of turbidity in acid medium [De La Rubia and Blasco, *Chemist-Analyst* **44,** 58 (1955)] failed to give reproducible results in our hands. In alkaline medium, however, we have observed that a much more reproducible type of turbidity can be developed. Both ethylenediamine-tetraacetic acid and formaldehyde are included in the medium, to react with calcium and magnesium, and ammonia, respectively, according to the suggestion of Berkhout and Jongen [*Chemist-Analyst* **45,** 6, (1956)]. In one of our procedures, 1 ml. of a solution containing approximately 0.008 to 0.030 mg. of potassium is added, with shaking, to 2 ml. of an alkaline mixture containing tetraphenylboron, ethylenediamine-tetraacetic acid and formaldehyde. Volume is adjusted to 6.0 ml. with 0.8% saline, and absorption measured at 420 mμ (Coleman Jr. spectrophotometer). Calculation of potassium content is accomplished by reference to a calibration curve, after correction for the reading of a suitable blank. Analysis of diluted urine and of solutions of ashed food or feces by this simple procedure has given results

agreeing well with the results of analyses by the flame photometer. Serum analysis, on the basis of use of tungstic acid filtrate equivalent to 0.1 ml. of serum, also gives excellent results which are, however, 5 to 10 per cent higher than those by the flame photometer. When allowance is made for this deviation, turbidity analysis appears to be admirably suited to quick detection of hypokalemia or hyperkalemia, when the flame photometer is not easily available.

10

Veränderungen der Nierenarbeit bei der Nephrolithiasis. F. Menne (Physiologisch-Chemisches Institut der Universität Münster, Germany)

Unter den Ursachen, die zur Bildung von Nierensteinen führen, spielen vegetativ nervöse Einflüsse eine entscheidende Rolle. Erregungen des sympathischen Nervensystems können Durchblutungsstörungen des Nierenparenchyms bedingen. In Tierversuchen wurde bei experimentell herbeigeführter Nierensteinbildung eine verminderte Durchblutung der Nieren nachgewiesen. Zur Klärung der Frage, ob beim Menschen die Nephrolithiasis mit Durchblutungsstörungen und allgemeinen Veränderungen der Nierenarbeit einhergeht, wurden Clearanceuntersuchungen ausgeführt. Mit Hilfe der kombinierten Inulin-p-Aminohippursäure (PAH)-Clearance, wurde die Nierenfunktion bei Nierensteinpatienten mit klinisch und röntgenologisch gesicherter Diagnose untersucht. Ergebnis: Die Durchschnittswerte aus 24 Bestimmungen betrugen für die Inulin-Clearance 136 ml./min./1.73 m.², die PAH-Clearance 462 ml./min./1.73 m² und die Filtrationsfraktion 0.31. Es ergaben sich also für die Glomerulusfiltration etwa normale Werte, während der Nierenplasmastrom deutlich eingeschränkt war. Bei 8 Patienten wurde die Clearance mehrere Wochen nach der operativen Entfernung der Steine wiederholt. Dabei zeigte sich, daß die durch Steine hervorgerufenen Stauungserscheinungen die PAH-Clearance deutlich beeinflussen. So hatte ein Patient mit einem tiefsitzenden Ureterstein eine mittlere PAH-Clearance von 388 ml./min./1.73 m.². Nach der Steinentfernung durch Ureterotomie stieg die PAH-Clearance auf einen Wert von 593 ml./min./1.73 m.² an. In weiteren 3 Fällen wurde die PAH-Clearance nach der operativen Steinentfernung gebessert, jedoch in keinem Fall normalisiert. Bei den restlichen 4 Patienten war die Nierenfunktion so nachhaltig geschädigt, daß die PAH-Clearance nach der Operation nicht anstieg. Bei allen Patienten blieb die PAH-Clearance eingeschränkt. Der verminderte Nierenplasmastrom bildet offenbar eine wesentliche Voraussetzung für die Rezidivierung der Steinbildung.

Symposium on Blood Electrolytes: II

E

Electrolyte disturbances in acute uremia. J. Hamburger (*Hôpital Necker, Paris, France*)

In the hope of obtaining information as to the part played by various electrolyte disturbances in the clinical picture of uremia, the following substances were estimated in 60 anuric patients both before and after dialysis with an artificial kidney: sodium, potassium, calcium, magnesium, chloride, bicarbonate, sulfate,

and phosphate. The comparison of clinical results and biochemical findings confirms the fundamental role played by electrolyte disturbances in the phenomena of acute uremia.

A fall in the plasma level of *sodium* is found only when water intake has not been strictly limited. In such cases, the deuterium space is increased and clinical symptoms are those previously described in cellular overhydration, asthenia, hypothermia, anorexia, nausea, vomiting, cramps, headache, and even convulsions and coma; electroencephalographic tracings in some patients showed generalized symmetrical slow waves. All these symptoms disappear after correction of the water and sodium changes.

Alterations in plasma level of *potassium* occur irregularly; their consequences were already well known before the present work, which confirms the accepted beliefs.

A fall in the plasma level of *calcium* is constant; but no known clinical symptom of acute uremia appears to be related to hypocalcemia.

An increase in *magnesium* is invariably found. It seems sometimes responsible for clouding of consciousness and for a prolongation of the QTs in the electrocardiograph.

The alterations in *anion equilibrium*, low chloride and bicarbonate, high phosphate, high organic acids, and very high sulfate levels, may be of greater importance clinically than has hitherto been recognized, as their correction by the artificial kidney appears to be closely related to the improvement of the clinical condition.

F

Blood electrolytes under the influence of cortical hormones. R. Neher (*CIBA Limited, Basle, Switzerland*)

A survey of the electrolyte and water distribution in the body shows the various places where the action of the cortical hormones should be followed. For a valid assessment of this action, it must be remembered that synergistic and antagonistic activities are brought about simultaneously by other hormones. The qualitative effects are furthermore markedly dependent on the physiologic or pathologic state of the organism, on the dose of the cortical hormone as well as the method and duration of the administration, the simultaneous supply of salt and water, the duration of the experiment, and other factors. After their recent chemical developments are surveyed, the activity of the cortical hormones on the metabolism of electrolytes in normal humans and Addisonians is analyzed in the light of some selected examples. Besides the renal effects as reflected in the blood serum and urine several extrarenal effects of clinical importance are discussed, e.g. those manifested in the sweat and saliva. After the "genuine" adrenal cortical hormones, desoxycorticosterone (cortexone), aldosterone, 11-dehydrocorticosterone, corticosterone, 17-hydroxycortexone, cortisone, and hydrocortisone, the effects of recent synthetic analogs of these hormones and of ACTH are dealt with. Finally a few clinical syndromes caused by hyperfunction of the adrenals resulting in disturbed electrolyte metabolism are discussed.

G

Fluid compartments and the excretion of electrolytes. B. Josephson (*The Central Clinical Laboratory, St. Eriks Hospital, Stockholm, Sweden*)

During the last two or three years several investigations have been published concerning the influence on the diuresis of experimental and clinical changes of the volumes of the different fluid compartments of the body. It has been found that even the infusion of isotonic solutions can give rise to an increase of the diuresis. These experiments are briefly reviewed and the respective theories critically discussed on the basis of observations made by a group at St. Eriks Hospital, Stockholm.

Only a few of the previous investigators in this field have been interested in the influence of experimental and clinical changes of the fluid compartments on the electrolyte metabolism. Some recently published observations on the excretion of Na, K, Cl and creatinine together with observations made by the St. Erik group show that changes in the volumes of the fluid compartments may be of influence on the excretion of Na and Cl, while K seems to be more independent. On the other hand the K excretion may be of influence on the creatinine clearance. The previous and the new results are briefly reported and discussed.

Some pitfalls in the methods of estimation of the kidney functions are pointed out.

SESSION 4. Urinary Constituents

11

Urinary sulfur partition in normal men and cancer patients. Daphne Papadopoulou (*Greek Cancer Institute, Athens, Greece*)

The partition of total sulfate, inorganic sulfate, total and neutral sulfur has been investigated in urine of 10 normal and 34 cancer patients. The total and inorganic sulfate is determined in the aliquots of urine filtrate after having been freed from phosphate according to the method of Fiske, the inorganic sulfate before and the total after hydrolysis with hydrochloric acid. The total sulfur is determined in aliquots of urine of filtrate after digestion with Pirie's reagent. The excretion in urine of total to neutral sulfur has also been estimated.

The mean value of the total sulfur of cancer patients has been found to be 54.2 mEq. and the neutral sulfur 17.12 mEq. The mean value of normal persons is 52.5 mEq. for total sulfur and 7.85 mEq. for neutral sulfur. The ratio of neutral sulfur to total sulfur is 15 in normal persons and 31.5 in cancer patients.

12

Blood and urine pepsinogen-like substances in children in health and disease. H. G. Grayzel, B. Elkan, L. Schneck, and J. Garza. (*The Departments of Pediatrics and Biochemistry, Jewish Hospital of Brooklyn, Brooklyn, N. Y.*)

There is experimental and clinical evidence that blood and urine pepsinogen may serve as indirect indices of hypothalamic-pituitary and adrenal cortical functional state. Studies were made of infants and children under normal conditions and conditions of stress (such as infection and operation) and before and after hydrocortisone and prednisone therapy. In addition, similar studies were

performed in diabetic patients. In general, there were significant rises in blood and urine pepsinogen values with increased hypothalamic-pituitary and adrenal cortical activity and in the case of the diabetic with the increased duration of the disease.

13

Urinary peptide excretion in the burned patient. B. Balikov, R. A. Costello, and E. R. Lozano (*Biochemistry Section, Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas*)

The urinary peptide loss was followed on 38 seriously burned patients, 16 of whom did not survive. Burned patients typically show an elevated peptide excretion until they are essentially healed. This excretion usually follows a pattern of "rhythmic spiking," such spikes lasting only a very few days and then quickly subsiding. The more serious burns characteristically have higher and more frequent spikes and take longer for the peptide excretion to drop to normal. On patients who recover, the initial spike is usually the highest, but patients whose condition deteriorates normally show successively higher spikes.

Complicating conditions which were accompanied by increased peptide excretion were: septicemia, surgical procedures, gastrointestinal bleeding and excessive wound purulence. Bed rest, varied protein intake, and uncomplicated temperature rises had no effect on peptide excretion. Growth hormone has an equivocal effect and intravenous protein hydrolysate results in a marked increase in excretion.

The theory is proposed that the source of peptides in the urine is endogenous and that hyperpetiduria is a reflection of tissue destruction or cellular disorganization. It is suggested that the assay of urinary peptides may serve as an objective laboratory aid to a physician in his evaluation of the condition of a burned patient.

14

The determination of amino nitrogen in urine. Loretta Langen, K. H. Slotta, G. T. Lewis, and J. H. Ferguson (*Departments of Biochemistry and Obstetrics and Gynecology, University of Miami School of Medicine, Coral Gables, Fla.*)

Current interest in the metabolism of proteins and of the individual amino acids requires rather frequently the determination of amino acid nitrogen in the urine. Methods which serve for the measurement of this value in the blood are not applicable directly because of the ammonia and urea present in the urine. One of the most specific methods devised to date [Van Slyke, MacFadyen, and Hamilton, *J. Biol. Chem.* **150,** 251 (1943)] involves heating in a closed tube at 100° and measurement of the CO_2 formed. Closed tube heating is time consuming for routine work and gas analysis has never been popular in clinical laboratories.

The procedure described provides for the removal of ammonia in vacuo from a solution alkalinized by an excess of magnesium oxide and determination of the color produced in the filtrate with β-naphthoquinone-4-sulfonic acid.

Recovery of individual amino acids and the influence of the presence of amino acids in combination is discussed.

15

The aminopolysaccharide of human urine and its excretion in disease states. C. Rich and N. DiFerrante (*Hospital of The Rockefeller Institute for Medical Research, New York, N. Y.*)

Urinary aminopolysaccharide precipitated with cetyl trimethyl ammonium bromide from normal human urine [DiFerrante and Rich, *J. Lab. and Clin. Med.*, (in press)] was found to be a mixture of at least two electrophoretically distinct proteins and an aminopolysaccharide. After digestion with trypsin and papain, no glucuronic acid containing material would pass through cellophane by dialysis. The undialyzable material was treated with amyl alcohol and chloroform, passed through a kaolin column and precipitated at 2° for 16 hours after addition of 3 volumes of ethanol saturated with NaCl. This resulted in the collection of 140 mg. of purified urinary aminopolysaccharide from 23 L. of urine. Its mobility during paper chromatography and electrophoresis was indistinguishable from that of chondroitin sulfate prepared from bovine nasal septa. Analysis demonstrated nitrogen 3.91%, sulfur 5.65%, uronic acid 39.7% and hexosamine 31.7%, of which 90.0% was galactosamine and 9.8% glucosamine. Ultracentrifugation resulted in the demonstration of a single broad peak with a sedimentation coefficient of 0.9 ± .3 S. The average anhydrous molecular weight calculated from the sedimentation data was 10,000. The purified aminopolysaccharide and chondroitin sulfate had the same infrared spectra except for minor variations in intensity, and were depolymerized by testicular hyaluronidase at similar rates. The optical rotation of the urinary compound was $[\alpha]_D^{27} = 18.3 \pm 2.0°$.

These data indicate that the material measured by the published procedure is closely similar in its composition and properties to chondroitin sulfate. The normal urinary excretion of this substance has been determined and, as well, the variation in a number of diseases. The significance of its excretion in several abnormal states is discussed.

16

The use of enzymatic tests for the detection of glucose in urine. J. J. Moran, P. L. Lewis, J. G. Reinhold, and F. D. W. Lukens (*William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa.*)

A simple color test for glucosuria which utilizes glucose oxidase and horseradish peroxidase in the presence of *o*-tolidine was recently described by Keston. Paper strips infiltrated with this enzyme system may be used to test presence of glucose merely by dipping into urine. Formation of any blue (or green) color indicates the presence of glucose.

The sensitivity and specificity of two preparations, Clinistix (Ames) and TesTape (Lilly), were tested with known solutions of various sugars in water and urine. Both glucose oxidase preparations were shown to be highly specific for glucose. An evaluation of the enzyme technic was made by comparing its results with those of the copper reduction methods. Tests were run on 2087 urines, 512 obtained from miscellaneous patients (mainly negative for glucose) and the remainder from the diabetic clinic (30 per cent of the latter contained

glucose). In the miscellaneous urines, the classification of positive or negative was the same by both enzyme and copper reduction methods in 95 per cent. In the diabetic group the classifications were identical in 85 and 89 per cent respectively in two series.

Attempts to apply the glucose oxidase preparations to quantitation of glucose in urine were less satisfactory. For this purpose the Nelson-Somogyi blood sugar method was used as adapted to measurement of urine sugar by Reinhold and Thompson. Clinical evaluation of the results of glucose oxidase tests must take into account the higher sensitivity of this method as compared with copper reduction methods.

17

A simple specific test for urine glucose. A. H. Free, E. C. Adams, Mary Lou Kercher, Helen M. Free and Marion H. Cook (*Miles-Ames Research Laboratory, Elkhart, Ind.*)

A simple qualitative test for urine glucose has been devised which is based on the specific activity of the enzyme glucose oxidase. This enzyme catalyzes the oxidation of glucose by oxygen to yield gluconic acid and hydrogen peroxide. The enzyme and an appropriate indicator system are conveniently incorporated into a carrier such as a strip of filter paper. Orthotolidine-peroxidase is a suitable indicator system which gives a blue color in the presence of hydrogen peroxide. A test is readily accomplished by merely dipping the impregnated stick of filter paper in a specimen of urine. Color development in the presence of glucose occurs within 1 minute. Application of the test to several thousand urine samples has demonstrated the complete specificity for glucose under the conditions of testing. The test will detect glucose concentrations of less than 0.1%. Quantitation of glucose in urine has not proved to be practical by paper strip methods which employ enzymes.

18

Detection and determination of sugar in the urine. F. B. Moreland (*Department of Biochemistry, Baylor University College of Medicine and Veterans Administration Hospital, Houston, Texas*).

The new materials for use in testing urine, Clinistix and TesTape, are much more specific for glucose than are the traditional reducing reagents, such as Benedict's qualitative reagent or Clinitest. They have the advantage of simplicity of application; the test can be done quickly and easily with no additional equipment. However, they have the disadvantage of missing other glycosurias than *glucosuria*. If the excretion of galactose, fructose, pentose, lactose, etc., is to be detected, a less specific test should be used. If, however, one of the new tests were used on all urines which show a positive reducing test, it would be quickly established whether the positive reducing test was due to glucose. The present investigation was undertaken to compare the two glucose oxidase tests, to verify their sensitivity, and to evaluate the semiquantitative interpretation of one of them (TesTape). Clinistix and TesTape are both more sensitive than Benedict's test or Clinitest. Data will be presented on the qualitative response of Clinitest and TesTape to varying concentrations of urine sugar as shown by quantitative determinations.

19

The significance of melituria in pregnancy. M. Gross, and R. Sexton (*The Margaret Hague Maternity Hospital, Jersey City, N. J.*)

The adjustments imposed by gestation may accentuate any latent or overt disordered patterns of metabolism. Thus the pregnant state presents a useful period for biochemical investigation. To meet the nutritional demands of the developing fetus, there must be adequate utilization of carbohydrate by the mother. In the event that is not possible, as in the diabetic or prediabetic, this factor may contribute to a number of obstetric complications such as large fetal size, abnormal placental function, toxemia, congenital anomalies, and fetal wastage.

The early recognition of deranged handling of carbohydrate is therefore of great importance, and continuous routine examination for sugar is a mandatory part of prenatal care. Normally there may be occasional melituria in pregnancy as a result of lactose excretion associated with the preparation for lactation, or an apparent decrease in the renal threshold for glucose. The usual differential urine sugar tests are not easily performed on a routine screening basis. However, the availability of enzyme-impregnated test papers which react specifically with glucose makes this differentiation possible.

In this study periodic urine examinations were made in almost 1000 pregnancies. Reducing substance (Benedict's), glucose (glucose oxidase-peroxidase-impregnated test paper) and acetone were determined and the results evaluated in relation to weight, parity, duration of pregnancy, diet, family history of diabetes, weights of previous babies, and the obstetric outcome of the present pregnancy. In addition, patients with true glycosuria were given a glucose tolerance test. A correlation of findings is made and their significance discussed.

20

Observations on the Evans blue dye method for determining blood volumes. J. K. Kirby, C. F. Pelphrey, and J. R. Rainey, Jr. (*Drs. Pelphrey and Rainey Clinical Pathology Laboratories, and The Institute for Clinical Research, Austin, Texas*)

Since 1944 when Gregerson published his Evans blue dye method for determining blood volume, there has grown in the literature a mass of data concerning all aspects of the determination. Much of the literature has been critical, and from a theoretical standpoint such criticism may be justified. However, much of the criticism is obviated by the applicability of the dye method to routine clinical use. It is the purpose of this paper to present results with the dye method in three small hospitals, and to show that the method is reliable enough for clinical use yet simple enough that it can be performed in any clinical laboratory.

In this study the method has been modified so as to use only a small amount of dye. Such modification has had no effect on the precision of the method, while the danger of staining the patient has been diminished or eliminated. Present studies include the use of capillary blood for determinations of hematocrit and dye concentration. Selection of a set of normal values presented some difficulty. Basing normal values on a ml./kg. body weight basis is subject to error

in obese patients. Our normal values for plasma volume, blood volume, and red cell volume are based on tables calculated from height, sex, and frame size.

The application of the test as we have performed it routinely is discussed for the following conditions: traumatic shock, chronic shock, continual slow hemorrhage, polycythemia, dehydration, and following transfusion therapy.

We hope these studies will encourage other smaller institutions to use this rapid, simple, inexpensive, and clinically reliable determination of total blood volume.

SESSION 5. (A) Normal Values

21

Some normal laboratory values in the dog. J. E. Baer, H. M. Peck, and S. E. McKinney (*Merck Institute for Therapeutic Research, West Point, Pa.*)

Although extensive use is made of dogs in laboratory work, there is in the literature little systematic information concerning clinical chemical norms in this species. As a part of preclinical evaluation of potential drugs in this laboratory, a number of the customary clinical chemical tests are performed in mongrels over periods of several months: total urea nitrogen and creatinine excretion, plasma protein and nonprotein nitrogen, glucose, urea, serum bilirubin, bromsulfalein, electrolytes, etc. Some of the data that we have accumulated on the range of normal values in drug-free animals will be reported, with relevant citation of the literature as it pertains to this species.

For most clinical tests the range of values resembles that for humans. Outstanding differences include the distribution of electrolytes between erythrocytes and plasma, failure of the cephalin floccuation test, absence of acetylation of sulfonamides, and some differences in renal function and renal transport mechanisms.

Hematologic data obtained in normal dogs include cell counts, hemoglobin, hematocrit, sedimentation rate, and prothrombin time. The frequency of abnormal findings in the mongrel will be evaluated.

22

Further studies of racial differences in serum gamma-globulin concentrations. W. H. Long, R. Nassif, V. Yonan, and J. G. Reinhold (*William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa.*)

Healthy North American Negroid blood donors have been found to have significantly higher serum γ-globulin concentrations than Caucasoid blood donors living in the Philadelphia area. These differences were considerable when the zinc turbidity method of Kunkel was used. Smaller differences, although still statistically significant, were observed when zone electrophoresis or ammonium sulfate turbidity measurements according to de la Huerga and Popper were done. Studies have been made of the flocculum formed by addition of zinc sulfate to sera of Negroids and Caucasoids in the presence of barbital buffer at *p*H 7.50. The flocculated protein, dissolved with the aid of ethylenediamine tetraacetate, was applied to filter paper strips and subjected to electrophoresis at *p*H 8.6 in

barbital buffer. Distinct differences were observed between material derived from the sera of Negroid and Caucasoid donors. In the latter the precipitate was composed mainly of γ-globulin with some β-globulin. That from the Negroids, in addition to larger amounts of γ- and β-globulin, nearly always included a distinct α_2-globulin zone so that it was possible to recognize in most instances the race of the donor from the appearance of the electrophoretic pattern of the zinc flocculum. The effect of varying *p*H and other conditions of precipitation will be discussed. Substantial amounts of carbohydrate staining material is present in the α_2-globulin component of the zinc flocculum.

23

The variation with age of the concentration of some constituents in serum from normal infants. B. Josephson (*St. Eriks Hospital, Stockholm, Sweden*)

In 276 healthy infants, newborn and up to 6 years of age, the serum concentrations of Na, K, Cl, Ca, total protein, protein fractions, and cholesterol were determined. The subjects were divided in groups according to age and the results were statistically treated. The means of the analysis results plotted against the age of the subjects will be demonstrated. Several significant changes with age were observed, especially in K concentration and in the α_2- and γ-globulins.

24

Human serum bilirubin: an immediate method of determination and its application to the establishment of normal values. J. E. O'Hagan, Teresa Hamilton, H. G. Le Breton, and A. E. Shaw (*Red Cross Blood Transfusion Service, Brisbane, Queensland, Australia.*)

The technic of Powell [*Amer. J. Clin. Path., Tech. Sect.* **8,** 55 (1944)] has been modified for the determination of the serum bilirubin of apparently normal blood donors, by reading optical density of the solutions immediately after mixing the reagents and using a standard based on a more appropriate solution of crystalline bilirubin in pigment-free serum.

Seven specimens of bilirubin were examined spectrophotometrically, the disproportionality of the millimolar extinction coefficients at 453 mμ for the free bilirubin and at 532 mμ for the azobilirubin complex indicated the presence of an unreactive yellow pigment.

The destructive effect of sunlight on solutions of bilirubin, noted in the literature, was confirmed, hence all estimations were done without delay, or exposure to light for any length of time. The results obtained in 1953, on 5540 apparently healthy Brisbane blood donors, to detect possible carriers of homologous serum hepatitis, revealed no definite correlation between previous history of jaundice and increased serum bilirubin values, but as a precautionary measure individuals with values above 1.5 mg. per 100 ml. were not accepted as donors.

When 200 random values were plotted, the distribution curve gave a mean value of 0.417 for the values less than 1.0 mg./100 ml. The 133 male values in this group gave a mean of 0.448, and the 67 female values a mean of 0.363. This lower value for females is not unexpected in view of the lower blood hemoglobin content. Furthermore, only two of the 53 donors, who among the 20,000 screened

had bilirubin values greater than 1.5 mg. per 100 ml. were females, and one of these was subsequently diagnosed as having cholelithiasis.

25

Effect of storage of blood on hepatic tests. V. L. Yonan and J. G. Reinhold (*William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa.*)

A study has been made of the stability of the components of blood serum related to several of the hepatic tests. Serum was separated from cells within 4 hours after collecting blood. Aliquots tested immediately and again after storage at 5° overnight showed no difference in thymol turbidity, cephalin-cholesterol flocculation, and 1-minute direct and total serum bilirubin measurements. However, a significant lowering of the zinc sulfate turbidity and phenol turbidity readings occurred. A second series of experiments differed only in that the serum was allowed to remain in contact with cells overnight. Zinc turbidity and phenol turbidity remained unchanged. In a third series, true serum was obtained by collecting the blood anaerobically and allowing it to clot under mineral oil. The serum was preserved overnight at 5°. The zinc and thymol turbidities tended to increase slightly while serum bilirubin concentrations tended to decrease.

The results indicate that little change will occur in thymol turbidity and cephalin cholesterol readings if blood or serum is held at 5° for 24 hours. After 120 hours, a trend toward lower readings was evident. Zinc turbidity and phenol turbidity measurements, however, were affected by the method of collecting blood and subsequently by the method of storage. The peculiar behavior of the zinc turbidity test appears to be related to the carbon dioxide content of the serum.

SESSION 5. (B) Instrumentation

26

Adaptation of macro methods to micro analysis. A. R. Slonim, (*Lynn Hospital, Lynn, Mass.*)

Microchemical analysis is important not only in those cases in which it is difficult to obtain sufficient blood or complete a battery of tests, but also for duplication in testing in the event of laboratory errors. Therefore, a flexible program is proposed for converting existing macro methods to micromethods.

Existing standard procedures should be examined for the possibility of proportional reduction of the quantities of substances used for any given test. For methods determined colorimetrically, it is important to know first the minimum cuvet volume for each colorimeter in use. The importance of measuring micro amounts with appropriate glassware is stressed. An important consideration in any test is the calibration curve with its relationships: slope, colorimeter sensitivity range, and extent to which it obeys Beer's law. Dilutions are not indicated in those tests where the calibration curve departs from Beer's law or falls in the high sensitivity error of the colorimeter used. To a small extent certain colorimeters or spectrophotometers are more suitable than others for particular procedures. In certain cases the limitations in converting to a micro test warrants the adoption of an entirely new micro method. Generally, the majority of tests

appear to allow for a flexible program in which calibration tables apply equally to procedures utilizing 1 ml. or 0.1–0.2 ml. of blood or serum.

27

A single automatic buret for ultra micro, micro, and macro titration. Samuel Natelson, (*Rockford Memorial Hospital, Rockford, Ill.*)

A buret will be demonstrated and discussed which is capable of practical titration of volumes of from 0.1 to 10 ml. with an accuracy of at least one part in 100 for all volumes. The limitation of accuracy is essentially the sharpness of end-point recognition.

A precision glass plunger moves through a teflon gasket of special design, to prevent leakage, into a chamber containing the liquid to be delivered. The displaced liquid is delivered below the level of the liquid being titrated which is agitated either by a stream of air or with a magnetic stirrer.

The movement of the plunger is measured by means of a dial indicator attached to a metal rod which moves with the plunger. The metal rod is held in place by means of a spring catch. When titration is completed the spring is released, the rod returning to its original position and the dial returning to zero. This automatically sets the instrument for the second titration. On the dial 100 divisions represent 0.1 ml. The dial rotates 25 times so that up to 2.5 ml. may be delivered before the catch need be released. Continuous titration up to 10 ml. may be achieved by releasing the catch four times. Thus the buret combines ultramicro, micro, and macro titration in one buret.

28

An automatic method for colorimetric analysis. L. T. Skeggs, Jr. (*Department of Pathology, School of Medicine Western Reserve University, Cleveland, Ohio*)

The increasing number of laboratory examinations used by clinicians to arrive at more accurate diagnoses has placed a tremendous burden on the biochemistry laboratories. Many new analyses have come into use which are both time consuming and technically difficult. It therefore seemed that an automatic machine would be of great value, were it capable of performing those routine chemical procedures which still require the majority of technical time, laboratory space, and glassware.

A machine has been devised which is generally applicable to colorimetric methods of analysis. Samples of whole blood, serum, or urine are introduced by means of a pump and are propelled through a small continuous dialyzer together with a constant proportion of a suitable diluent. The substance to be determined is transferred in the dialyzer to a flowing stream of a reagent which is then continuously processed to produce a specific color change. The degree of color change and therefore the concentration in the original sample is measured and recorded by passing the flowing stream through a recording flow-cell colorimeter. Successive samples may thus be run through the machine at intervals of 2 or 3 minutes, allowing a total of 20 to 30 analyses per hour.

The automatic analyzer has thus far been adapted to the determination of urea, calcium and glucose. The analytical results compare favorably with those obtained by conventional methods.

29

Spectrophotofluorometry: A new tool for analysis at the submicrogram level. D. E. Duggan and B. Vasta (*Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Bethesda, Md.*)

A spectrophotofluorometer capable of high-intensity monochromatic activation at any wavelength throughout the quartz-ultraviolet and visible regions of the spectrum and of measuring the resultant fluorescence spectrum has previously been described [Bowman *et al.*, *Science* **122,** 32 (1955)]. Two commercial versions of this instrument have subsequently become available and have been applied in this laboratory to the routine analysis of many compounds of pharmacologic and biochemical interest. All three instruments consist essentially of the same components: A high-pressure xenon arc source emitting a continuum from 200–800 mμ; two monochromators, one for the selection of monochromatic activation, and the second, at right angles to the first to analyze the resulting fluorescence; and a nine-stage photomultiplier to detect the emitted light. Both activation and fluorescence spectra may be rapidly visualized on a cathode ray oscilloscope or pen-and-ink recorder.

Tryptophan, 5-hydroxytryptamine (serotonin), tyrosine and tocopherol, all of which emit ultraviolet fluorescence, are routinely measured in this laboratory by spectrophotofluorimetric analysis. Details of the estimation of free and bound tocopherol in tissues are presented.

30

A single-piece cell for rapid analytical electrophoresis. G. Kegeles (*Chemistry Department, Clark University, Worcester, Mass.*) and W. Slavin (*American Instrument Company, Silver Spring, Md.*)

A cell of one-piece construction is described in which the boundaries are formed by layering the solution below the buffer. Reservoirs are supplied to permit the boundaries to be sharpened by withdrawing solution through a fine needle inserted in the boundary. This cell greatly reduces the time necessary for completing a formerly time-consuming phase of an electrophoresis experiment. Extremely sharp boundaries are expected. Data are presented to illustrate the economy of time in using this cell in conjunction with the prism electrophoresis technic. The general virtues of this method over those of conventional schlieren and zone methods are reviewed.

SESSION 6. Hematology

31

Clinical chemical analysis of hemorrhagic disorders. G. F. Lanchantin (*Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas*) and Arnold G. Ware, (*Department of Biochemistry, University of So. California School of Medicine, Los Angeles, Calif.*)

In the majority of hospitals in the United States the analysis of various blood clotting components of noncellular origin falls within the realm of the clinical chemistry laboratory. To date, the determination of coagulation components and reactions, such as calcium, fibrinogen, prothrombin, prothrombin consumption

and whole-blood clotting time, is a common occurrence. However, with the advent of newer knowledge concerning the complex clotting mechanism and the recognition of numerous hertofore-unknown hemorrhagic disorders, adoption of more advanced methods for clinical chemistry diagnosis has become necessary. This is particularly true in the different types of hemophilia and in cases of spontaneous bleeding where a clotting anomaly must be determined in order to facilitate better diagnosis and therapy.

This paper presents a stepwise series of qualitative and quantitative procedures whereby it is possible to determine the deficiency of an essential clotting factor or the qualitative activity of a circulating anticoagulant in any bleeding disorder of unknown etiology. The procedures outlined are well-adapted to rapid routine analysis utilizing reagents easily prepared in the laboratory. A number of bleeding dyscrasias in which this series of procedures has been found useful in determining the lack of an essential clotting component or presence of a specific type of anticoagulant are presented.

32

Role of blood proconvertin: Convertin as a possible factor in coronary atherosclerosis. J. Black, R. S. Wayne, and I. J. Greenblatt (*Messinger Research Laboratory, Beth-El Hospital, Brooklyn, N. Y.*)

The approach to atherosclerosis may be associated with defects in the coagulation mechanisms of the blood. This study was undertaken to evaluate proconvertin-convertin in patients with myocardial infarctions. One hundred patients with infarctions were randomly selected. Proconvertin-convertin was measured by the technic of Jensen *et al.* One hundred subjects clinically free of coronary atherosclerosis were used as controls.

33

The effect of simultaneous administration of vitamin K_1 and dicumarol on the prothrombin in rat plasma. A. L. Babson, S. Malament, G. H. Mangun, and G. E. Phillips (*Biochemistry Department, Warner-Chilcott Research Laboratories, Morris Plains, N. J.*)

No amount of menadione would counteract the hypoprothrombinemia action of 8 mg. dicumarol in rats. Vitamin K_1, however, was very effective. This confirms results of other workers. Data on the effects of simultaneous administration of dicumarol and vitamin K_1 have not been reported. The vitamin K_1-dicumarol antagonism is not a simple metabolite-antimetabolite competition. When the ratio of vitamin K_1 to dicumarol is held constant the hypoprothrombinemia goes through a maximum as the dosage is increased. At high doses the prothrombin activity returns to normal. The data suggest that at any one level of vitamin K_1 there is a maximum response attainable, and increasing the dicumarol intake above the level necessary to reach this maximum has no effect. It was thought that the variation in response to dicumarol therapy was due perhaps to variations in dietary vitamin K. If this were true, administering relatively large amounts of vitamin K along with the necessarily increased dose of dicumarol might eliminate this variation by rendering the dietary vitamin K negligible. When rats were maintained on a diet containing dicumarol and vitamin K_1 in

proportions that caused a hypoprothrombinemia in the therapeutic range, the variation in response from day-to-day and rat-to-rat was slight. A similar picture was seen when rats were maintained on a stock diet to which only dicumarol had been added at a concentration giving the same degree of hypoprothrombinemia.

34

Fetal hemoglobin: Studies of its resistance to alkali denaturation and a method for its determination. W. R. C. Golden, and W. M. Layton, Jr. (*Department of Laboratories, The Stamford Hospital, Stamford, Conn.*)

The discovery that an alkali-resistant hemoglobin, similar to fetal hemoglobin, is present in certain types of anemia has provided an additional method to aid in hematologic diagnosis.

The Singer method for the determination of this alkali-resistant fraction, while simple and easy to perform, is not sufficiently sensitive to permit accurate measurement of small but clinically significant amounts of the material.

The modification of the Singer method presented in this report provides an increase in sensitivity in the determination of alkali-resistant hemoglobin. This extends the range of the method to concentrations of fetal hemoglobin of less than 5% of the total hemoglobin.

This has been accomplished by carrying out the spectrophotometric measurements of oxyhemoglobin in the "Soret" region (400–420 mμ) of the spectrum. The absorption of the hemoglobin in this region is approximately 7 to 8 times greater than at 542 mμ. Comparison studies have shown the validity of this change.

Using umbilical cord blood as readily available material for investigating alkali denaturation of fetal hemoglobin, we have studied the effect of temperature, concentration of alkali, and concentration of hemoglobin on the rate of the denaturation reaction. Variations in technic for the preparation of a red-blood-cell hemolysate have also been studied and this part of the procedure simplified.

The method developed in this study has been applied to artificial mixtures of cord blood and adult blood to test the sensitivity and to verify the over-all reliability of the procedure.

35

Heterogeneity of protein in hemoglobin S. C. A. J. Goldberg (*William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa.*)

Staining of electrophoretically separated hemoglobin S by means of amidoblack 10B has revealed the presence of two protein peaks, although the gaussian curve of the unstained hemoglobin suggested homogeneity. In contrast, the hemoglobin A of these patterns contained only one protein component. Heterogeneity was observed in the hemoglobin S component of 3 patients with sickle-cell trait so that it is not an isolated phenomenon. Studies of other patients and other hemoglobins will be reported.

Symposium on Porphyrins

H

The properties, estimation methods, hematologic features and some other more general aspects of different abnormal human hemoglobins. T. H. J. Huisman (*Department of Pediatrics, State University, Groningen, The Netherlands*)

Since the discovery of Pauling (1949) that the sickling phenomenon of red cells in sickle-cell anemia is dependent on a pathologic variety of the hemoglobin molecule, many other abnormal hemoglobins have been described. At the present time up to eleven kinds of human hemoglobin are recognized. The discovery of the existence of these hemoglobins was made possible by methods based upon differences in some properties of the protein molecules. The most important technics are the Tiselius moving-boundary method, paper electrophoresis, chromatography on the cation exchanger Amberlite IRC 50, and salting-out procedures. The estimation of fetal hemoglobin may also be carried out by alkali denaturation methods and some other less sensitive technics. All these procedures are discussed in detail.

Next to the methods based on fundamental physicochemical procedures some clinical and hematologic data may be valuable for the determination of the presence of an abnormal hemoglobin. Apart from the recognition of the known abnormal derivatives of normal hemoglobin the mentioned methods may be valid for the investigation of other unknown abnormalities in the Hb molecule in any obscure hemolytic anemia in which diagnosis is not established by the conventional procedures. Some examples of such conditions are given. It is shown that even in clinically fully normal individuals an abnormal form of hemoglobin may be present.

Finally some genetic aspects of the hemoglobin abnormalities and the incidence of the different kinds of human hemoglobin in different parts of the world are discussed.

I

Bilirubin glucuronide, the direct-reacting bilirubin, in serum, bile and urine. R. Schmid (*National Institute of Arthritis and Metabolic Diseases, National Institues of Health, Bethesda, Md.*)

Since the introduction into clinical chemistry of diazotized sulfanilic acid for the determination of serum bilirubin, it has been recognized that bilirubin occurs in two different forms, one giving a so-called "direct" reaction, the other exhibiting an "indirect" reaction, i.e., the coupling takes place only after the addition of alcohol. In general, when the jaundice is due to excessive destruction of hemoglobin, as in hemolytic anemia, most of the bilirubin in serum is indirect-reacting. If the jaundice results from intrahepatic or extrahepatic obstruction to bile passage into the intestine, the bilirubin in serum is mostly direct-reacting. Various explanations have been offered to account for this clinically important difference, but the experimental evidence in support of these theories has been controversial. Recent studies have shown the direct-reacting bilirubin obtained

from serum or bile is water-soluble over a wide *p*H range, whereas indirect-reacting pigment is practically insoluble in water below a *p*H of 8. It has been suggested that the water solubility of the direct-reacting fraction may be the result of a conjugation of bilirubin with a polar substance. Because of their instability, satisfactory chromatographic purification of these pigments, and particularly of the direct-reacting fraction, has not been achieved.

This difficulty has now been overcome by studying the more stable dipyrromethene diazonium pigments, which were obtained by treating normal bile or serum and urine from jaundiced patients with an excess of diazotized sulfanilic acid, dissolved in dilute hydrochloric acid. In this reaction, one mol of bilirubin reacts with two mols of the reagent, yielding two mols of hydroxypyrromethene diazonium salt. After preliminary purification and butanol extraction, the azopigments obtained from serum, bile, and urine were separated and purified by ascending paper chromatography, employing a solvent system consisting of 75 parts methyl-ethyl ketone, 25 parts *n*-propionic acid, and 30 parts water. It was found with this system that direct-reacting bilirubin of jaundiced serum and almost all of the bilirubin in fresh bile and in jaundiced urine gave rise to an azopigment exhibiting an Rf of 0.25 to 0.30 (azopigment B). On the other hand, the azopigment obtained from indirect-reacting serum bilirubin, crystalline bilirubin, and heated bile exhibited an Rf of 0.45 to 0.50 (azopigment A). Diazonium salts of synthetic neoxanthobilirubinic acid and isoneoxanthobilirubinic acid were found to have an Rf identical with that of indirect-reacting serum bilirubin and of crystalline bilirubin. Absorption spectra in the visible range appeared to be identical for azopigments A and B.

Hydrolysis of repeatedly chromatographed azopigment B in 1 N hydrochloric acid for 1 hour at 100° resulted in its complete conversion to azopigment A. For each micromole of hydrolyzed azopigment B, one micromole of glucuronic acid was liberated, as determined by the carbazole method. Data of a typical experiment are given. Hydrolysis could also be achieved by incubating azopigment B with β-glucuronidase of animal or bacterial origin. Since fresh bile and jaundiced urine yielded almost exclusively azopigment B, and since two moles of hydroxypyrromethene diazonium salt are produced from one mol of bilirubin, it would appear that direct-reacting bilirubin is conjugated with 2 mols of glucuronic acid. In analogy with other instances of glucuronide formation, it may be assumed that the glycosidic linkage occurs at the α,α' hydroxy groups of bilirubin. The finding in bile and urine of minute amounts of azopigment A together with azopigment B suggests that small amounts of direct-reacting bilirubin may be present as a monoglucuronide. This observation is in agreement with an earlier report, indicating the separation of two closely related water-soluble bilirubin fractions from bile. While this work was in progress, Billing and Lathe published an abstract, in which they also suggest that in bile, bilirubin is excreted as an ester glucuronide.

In the serum of patients with regurgitation jaundice, conjugated bilirubin was found to predominate ("direct-reacting" bilirubin). On the other hand, in retention jaundice, most of the serum pigment was shown to be free bilirubin ("indirect-reacting" bilirubin). Since in jaundiced patients with bilirubinuria

the urinary bilirubin was found to yield almost exclusively azopigment B, it appears that the kidneys can only excrete conjugated bilirubin. In a child with congenital non-hemolytic jaundice, exhibiting 30 mg./100 ml. of free bilirubin in the serum, no bilirubin could be demonstrated in the urine.

These findings demonstrate that in the serum, glucuronic acid-*conjugated* bilirubin gives the direct van den Bergh reaction, whereas *free* bilirubin, because of its insolubility in water, requires the prior addition of alcohol to initiate the coupling with the diazo reagent. In the bile, most or all of the bilirubin is excreted as a water-soluble glucuronide. In regurgitation jaundice, conjugated bilirubin gains access to the blood and hence to the urine, resulting in bilirubinuria.

SESSION 7. Enzymes

36

Enzyme studies in the perinatal period. M. M. Friedman and B. Lapan (*Chemistry Division, Department of Laboratories, Lebanon Hospital, New York, N. Y.*)

Certain enzymatic analyses of the blood have been found to be useful in clinical diagnosis. Among these are tributyrinase, cholinesterase, and aldolase. Studies of these enzymes in relation to diseases of the newborn are scanty or nonexistent.

Since the normal ranges of enzyme activities in the newborn may vary from the levels in adults, it is necessary to establish the normal levels of tributyrinase, cholinesterase, and aldolase in the plasma of the newborn. The methods were modified and adapted to the small volume of plasma available from heel puncture.

The blood was drawn from heel puncture directly into heparinized capillary tubes, the tubes were sealed and centrifuged, and the cell/plasma ratio was determined. The plasma fraction was separated and analyses were carried out in single determinations or in duplicate whenever enough plasma was available. Specimens were taken during the first 6 days of the neonatal period and the results were divided into three groups; the first and second days, the third and fourth days, and the fifth and sixth days. The newborns were those delivered normally by mothers free of clinical disease. The normal ranges for these enzymes serve as a basis for the evaluation of results in disease states of the newborn.

A parallel study was carried out for enzyme activities in cord blood and mothers' blood drawn at the time of delivery.

37

Fermente des Glucose-6-phosphat-dehydrogenasesystems im Blutserum. F. H. Bruns (*Institut fuer Physiologische Chemie der Medizinischen Akademie, Duesseldorf, Germany*) Abstract to follow.

38

The effect of various therapeutic agents upon alkaline phosphatase levels. Eleanor Berman (*The Illinois Masonic Hospital, Chicago, Ill.*)

The increased and unexplained appearance of low values of alkaline phosphatase activity led to the investigation of the possible effect of commonly used pharmaceuticals upon this enzyme.

Quantities of phenobarbital, Seconal, acetyl salicylate, paraldehyde, penicil-

lin, streptomycin, erythrocin and Terramycin in the amounts corresponding to levels found in vivo during the therapeutic administration of these agents were added to sera and incubated. Alkaline phosphatase activity of sera so treated was compared with untreated sera. A modified spectrophotometric method of Fister, based upon the methods and modifications of Fiske and Subbarow, and Bodansky, was used in determining the alkaline phosphatase levels.

Changes in alkaline phosphatase activity in patients following administration of these various agents were also followed.

39

Improved method for prostatic acid phosphatase. R. J. Davis and E. Wood (*St. Joseph's Hospital, Tampa, Fla.*)

This method uses Fishman's *l*-tartrate inhibition [*J. Biol. Chem.* **200,** 89 (1953)] combined with Powell and Smith's modified Grifol's method of color production [*ABT* **III,** 23 (March 1955)]. The advantages of this technic consist in the reduced number of controls, the stability of the color, the absence of protein precipitation, and the conduct of the test with all reagents in a single tube so that there is no need for transfer of aliquots. The results agree with those reported using Fishman's standard technic.

REAGENTS. *l*-Tartrate buffer and citrate buffer as described by Fishman
Disodium phenyl phosphate substrate 0.2% aqueous solution
4-Aminoantipyrine 1.5% aqueous solution
Potassium ferricyanide 4% aqueous solution
Sodium carbonate-bicardonate solution (equal volumes of 4% aqueous solutions)

The procedure is set up in Coleman 19 x 105 mm. cuvets.

Cuvet	*Water*	*Citrate buffer*	*Substrate*	*Serum*	*Tartrate buffer*
A	4.2	0	0	0	0
B	0	2.0	2.0	0	0
C	0	2.0	2.0	0.2	0
D	0	1.6	2.0	0.2	0.4

Serum is added to C and D after warming to 37°. Incubate at 37° for 1 hour. Immediately upon removal from the water bath 0.2 ml. of serum is added to Cuvet B and 2.0 ml. carbonate-bicarbonate solution added to all tubes. Mix by inverting after each addition. Add 1.0 ml. each of aminoantipyrine and ferricyanide solution. Add 5 ml. of water to all tubes. Allow to stand 15 minutes or longer and read at 510 μ against A. The procedure is standardized against phenol and read in King Armstrong units. No tartrate blank has been included since we have found it unnecessary.

Total acid phosphatase (T) in King Armstrong units is C minus B. Prostatic fraction of acid phosphatase in King Armstrong units is (T) minus (D-B).

This simplified technic has worked well in over 100 tests and given results comparable to those using Fishman's standard technic. We consider 0.6 King Armstrong units the upper limit of normal and repeated values over 2.0 indicative of prostatic carcinoma. In one case with a large prostatic stone the total phosphatase was 4.5 and the prostatic fraction 1.8. The highest values we have seen in a prostatic carcinoma with widespread metastases are 455.0 units total and 445.0 units prostatic fraction.

40

"Acid" phosphatase in lipoidoses. J. J. Carr and L. R. Tuchman (*Departments of Chemistry and Medicine, The Mount Sinai Hospital, New York, N. Y.*)

In cases of Gaucher's disease and of the Niemann-Pick disease one finds an increase of the acid phosphatase level in the serum. The nature of this phosphatase has been studied, especially in respect to specific substrates and inhibitors. Its relationship to prostatic, erythrocytic and other cell phosphatases is discussed and the diagnostic implications of these findings are considered.

41

A simplified procedure for the determination of serum transaminase. F. W. Fales (*Department of Biochemistry, Emory University, Emory, Ga.*)

The finding of Wroblewski *et al.* that serum transaminase was elevated after myocardial infarctions and after liver cell damage has elicited considerable interest, but the procedures outlined are beyond the scope of the usual clinical laboratory. This paper presents a simplified procedure.

The reaction is run in the more favorable direction—i.e. glutamate + oxaloacetate → α-ketoglutarate + aspartate, equilibrium constant about 7; the excess oxaloacetate is converted to pyruvate; and the optical density of the alkaline 2-4-dinitrophenylhydrazones formed from the ketoacids is determined at a wave-length of 510 μ. The decrease in the density of the test solution compared to a control measures the extent of transamination (pyruvate gives a density 3.66 times that of α-ketoglutarate). The determination is made quantitative by including pyruvate and α-ketoglutarate standards. The rate of change of the oxaloacetate concentration is enhanced by an initial glutamate concentration 20 times that of oxaloacetate, so that 30 minutes incubation at 37° is adequate. Errors due to the instability of oxaloacetate are minimized by storing the solid acid in the refrigerator until immediately before the determination. Also Versene is included in the reaction mixture to prevent the catalysis of oxaloacetate breakdown by calcium and magnesium. Added aluminum salt, in excess, serves to inactivate the enzyme, to catalyze the conversion of oxaloacetate to pyruvate, and together with barium hydroxide, to precipitate the protein. Further details concerning the method and the results obtained are given.

42

A preliminary report on some extraneous factors that may influence serum glutamic oxalacetic transaminase level. Chi Che Wang and I. Appelhanz (*Winter V. A. Hospital, Topeka, Kans.*)

In the course of establishing a normal SGO-T curve, it was found that all fasting sera gave a lower value than that withdrawn following breakfast. One control and three experimental series consisting of high-protein, high-fat, and high-carbohydrate breakfasts were conducted on 20 subjects. A fasting blood was followed by breakfast and four more bloods withdrawn at definite intervals.

Unexpectedly even fasting values of the same individuals varied considerably. One subject varied from 5 to 36 units. In the majority of the subjects, all types of breakfasts brought about a rise in the SGO-T level. The maximum rise

after a high-protein meal was 27 units after 2 hours. The corresponding values following a high-fat breakfast was 11 units and 4 hours, and those of high carbohydrate were 12 units and 2½ hours. The maximum rise of the control series was 13 units after four hours.

Judging from the variations of fasting SGO-T values obtained from the same individuals on different days, factors other than disease and foods may also play a small role on the SGO-T level. Indications are that either activity or emotional disturbance or both may be responsible for the fluctuations. The above statement is based on the correlation between the fluctuations of the SGO-T values and the mental attitude or the activity of the subject observed.

Although the difference in the values seems insignificant, a change in 30 units or thereabouts could, in the borderline cases, mislead the physician in the diagnosis as well as in the treatment.

43

The clinical significance of alterations in serum transaminases. F. Wroblewski (*Memorial Center for Cancer and Allied Diseases, New York, N. Y.*)

Transaminases are enzymes which catalyze the reversible transfer of the α-amino group of an amino acid to an α-keto-acid resulting in the synthesis of another α-amino and another α-keto-acid. Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase are two enzymes which are widely distributed in human tissues, and have been demonstrated to be present in all human sera tested. Serum glutamic oxaloacetic transaminase (SGO-T) and serum glutamic pyruvic transaminase (SGP-T) may be measured chromatographically, spectrophotometrically, and colorimetrically.

SGO-T and SGP-T activity are not increased above the normal range as a result of injections, degenerative neoplastic, allergic, reactive, congenital disease states, or pregnancy unless there is associated injury of heart muscle, skeletal muscle, or liver.

Clinical myocardial infarction is followed by characteristic changes in the activity of SGO-T and SGP-T, with the peak occurring within 24 hours and the activity falling to normal within 2 to 5 days. The changes in SGP-T are appreciably less than those of SGO-T activity. The study of alterations in serum transaminase in patients with chest pain and/or equivocal cardiographic abnormalities contributes to the differential diagnosis of various cardiorespiratory disease states.

The measurement of SGP-T alterations has been found to be a useful tool in the diagnosis and study of acute hepatic disease and acute extrahepatic biliary obstruction, and appears to be more sensitive than SGO-T in reflecting acute hepatocellular injury. SGO-T alterations more sensitively reflect active chronic hepatic disease. By the simultaneous measurement of SGO-T and SGP-T activity, it appears possible in most cases to differentiate acute from active chronic liver cell injury, and by the use of quantitative and serial measurement of both enzymes to differentiate diagnostically the clinical types of hepatic disease.

The quantitative and serial alterations of SGO-T and SGP-T are sufficiently characteristic in various cardiac and hepatic diseases to assist in differential

diagnosis when the serum enzyme elevations and serial changes are correlated with the clinical settings.

44

Pseudocholinesterase as a routine test of liver function in the diagnosis and assessment of portal hypertension. A. H. Hunt and H. Lehmann (*St. Bartholomew's Hospital, London, England*)

We have used the estimation of pseudocholinesterase in the assessment of patients with portal hypertension since April, 1952. Though the actual level of serum albumin may be considered the most valuable liver function test, it does not reflect short-term changes. The initial readings of pseudocholinesterase level show a wide range for each patient, but the changes are much less "sluggish" than those of the serum albumin, and are often of great significance.

In *extrahepatic obstruction* (30 cases) the values are almost invariably within normal limits, i.e. above 55 units. (The normal range was seen to be 55–120 units measured by the Warburg manometric technic, 1 unit equaling 1 μl. CO_2 liberated by 1 ml. of serum in 1 min. at 37°.)

In *mild cirrhosis* (31 cases) the average levels approach those found in normals, and are almost all within the normal range. The patients are in a good clinical state before shunt operation, and there is little change in the pseudocholinesterase level after operation.

In *moderate cirrhosis* (61 cases) the average level is below the lower normal limit (48 units), there is an inclination to rise after shunt operations. Here it seems that the pseudocholinesterase level gives a better indication of the improvement in liver function than any other single biochemical test. It is more often of diagnostic value.

In *severe cirrhosis* (69 cases) all readings were below normal with an average of 34 units. If on observation a fall of pseudocholinesterase level is seen, the prognosis can be considered to be bad. A rise shows that the patient is likely to survive the operation, should it become necessary on account of hemorrhage or intractable ascites. The course of clinical improvement follows closely the continued rise of the pseudocholinesterase. The final average is comparable with that found in patients with mild cirrhosis (63 units).

45

The use of the aldolase activity test in the diagnosis of viral hepatitis. J. Hořejší, L. Mirčevová, R. Soušek, J. Vaněček (*Central Biochemical Laboratory of the University Hospital, Charles University, Prague, Czechoslovakia*) Abstract to follow.

46

Enzymatic determination of urinary histidine daily output as a pregnancy test. P. Soupart (*Department of Biochemistry, Faculty of Medicine, University of Brussels, Belgium*)

Increased histidine urinary output has long been recognized as a concomitant of pregnancy. Up to five years ago, reliable methods for amino acid determination were lacking, leaving this approach to pregnancy diagnosis unsatisfactory. The quantitative method of Moore and Stein for amino acid determination

gave us the opportunity for reopening this question. This method is too tedious and expensive to be of current use as a standard clinical laboratory method. On the basis of data obtained from chromatography it seemed interesting to work out an easier and cheaper method. This has been found in the specific enzymatic decarboxylation of histidine by a bacterial decarboxylase, followed through the Warburg manometric method. The enzyme is prepared as acetone powder from *Cl. welchii*, strain N.C.T.C. No. 6785, according to Gale and Epps. Activity on each amino acid for which a bacterial decarboxylase has been demonstrated is checked. Undesirable decarboxylase activity is controlled through variable duration of acetone treatment. 1- and 3-methylhistidine are not decarboxylated. The reaction is run at *p*H 4.5 on aliquots of 24-hour urines. When plotting values from decarboxylation versus chromatography, every experimental point locates in the $\pm 10\%$ area from the ideal correlation line (precision of manometric method: 5–10%). At the present stage of this study, no value higher than 200 mg./24 hr. in normal urine, and, on the other hand, no value lower than 200 mg./24 hr. in pregnancy urine have been observed in more than 100 cases. After careful collection of 24-hour urine specimen, the result can be obtained within 2 hours.

SESSION 8. Symposium on Standardization

K

Standardization in clinical chemistry. I. D. P. Wootton (*Post Graduate Medical School, University of London, England*)

This paper deals with the development of standardization as it has taken place in Great Britain. The most important early work was in the field of hemoglobin estimation. After considerable preliminary work, a scheme for distributing regular blood samples was organized in 1951. The dispatch of these specimens still continues and detailed intercomparisons have been accomplished between Great Britain and interested American laboratories.

In 1953, comparison of serum and blood analyses on the same specimens submitted to different hospitals showed very wide variation of results. These trials have been repeated and extended since, and the findings are discussed, including those of the International Trial.

A scheme to enable internal checking to be done in the laboratory is described which has enabled significant improvement in routine accuracy to be maintained. Experiments in this country with certified lyophilized serum samples are also discussed.

L

Standardization of methods in clinical chemistry. D. Seligson (*Division of Biochemistry of the Graduate Hospital and the Department of Biochemistry of the Graduate School of Medicine of the University of Pennsylvania, Philadelphia, Pa.*)

One of the most difficult tasks in clinical chemistry is to establish the absolute quantity of a constituent in a biological fluid. When these constituents occur in minute amounts and in the presence of numerous interfering substances, the classical analytical procedures involving isolation, weighing, or titration cannot be applied. Sensitive methods, mainly photometric, must be used. These

methods generally are based on the development of a colored reaction mixture containing the sample, which is compared to a similar one containing the standard solution. Since most of the available reactions are not specific or stoichiometric the calibration of a method or its "standardization" often is difficult and. therefore, requires rigid control of the reaction conditions. The differences between the sample and the standard solution may lead to complications.

Examples of problems commonly encountered are presented. The measurement of bilirubin in serum and the problems related to its standardization are discussed. In this analysis the presence of serum proteins affects the pH of the reaction mixture and the amount of color produced per unit of bilirubin. Comparison of the absorbency of the products of the diazo reaction, when serum is present, with the absorbency of the standard may lead to erroneous results. Means of eliminating such difficulties are presented. A similar study of the bromsulfalein method is presented in which interferences from protein binding, turbidity, hemoglobin, and bilirubin are demonstrated and eliminated, so that readings on serum may be accurately compared with those on pure solutions.

The resolution of some of these problems through the use of a "standard" serum is discussed.

M

Suggestions for standardization in clinical chemistry. M. Guillot (*Laboratoire de Chimie Biologique, Faculte de Pharmacie, Universite de Paris, France*) Abstract to follow.

N

Ultramicro methods and standardization of equipment. M. C. Sanz (*Central Laboratory, University Hospital, Geneva, Switzerland*)

The general problems concerning biochemical analyses in sample volumes of 1 to 50 μl. are discussed. Actually most of the proposed ultramicro methods require specially trained personnel and often highly specialized equipment. In order to find broad application in the routine laboratory the equipment must be sturdy, easy to handle and to clean, permitting precise and fast work. Being as standardized as possible it must also be versatile, suitable for a great number of different determinations.

A series of new basic ultramicro equipment is presented, covering the fields of sample collecting, sample pipetting, precise automatic reagent delivery, colorimetric cuvets, ultramicro burets, and *p*H measurement.

Problems involved in titration methods, such as stirring, dilution errors, objective end-point determination (potentiometric, colorimetric) are discussed.

A new glass electrode is demonstrated for the accurate bedside determination of blood *p*H.

SESSION 9. Lipoid Analysis and Lipoproteins

47

Improved clinical equipment for ionophoresis on supported electrolytes. R. Jonnard and F. J. Scalera (*The Paterson General Hospital, Paterson, N. J.*)

A versatile ionophorometer with a plurality of simultaneously usable cells is described. The apparatus permits the rapid routine determination of elec-

trophoretic patterns, electromigration velocities, isoelectric curves, and other related data either singly or in various combination—and, if necessary, simultaneously—on several specimens. Features are incorporated allowing much higher effective potential gradients, for a given applied voltage. It is also possible to combine the effects of the electrical field with gravity or a chromatographic gradient, in either uni- or bidirectional migration schemes. The non-electronic power supply, based on a new concept, is the culmination of a critical study of rather general problems of voltage and of current stabilization encountered in biochemical and biophysical experimentation. It is fully described. One important feature of this device is that it may serve the additional purpose of powering either a scanning analyzer or a data-computing system when such items are required, without undue multiplication of the equipment.

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The determination of blood cholesterol and its esters by the anthrone method. R. Jonnard and F. J. Scalera (*The Paterson General Hospital, Paterson, N. J.*)

In a search for a convenient, yet fully reliable clinical method for cholesterol analysis, a thorough study of the anthrone-glucoside colored compound was made. The details of the routine procedure adopted are given. The initial isolation of cholesterol and the separation of the esters are still based on the Sperry-Schoenheimer digitonin precipitation. The subsequent technic of color development with anthrone in acid-aqueous solution is based on an evaluation of spectrophotometric isobestic curves.

The method is simple, the reagent preparation requiring neither undue time, care or accuracy, and the procedure is reproducible and reliable in the hands of a relatively skilled technician. A short statistical analysis is included.

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Rapid colorimetric micro method for analysis of neutral fats and fatty acids in biological materials. R. Jonnard (*The Paterson General Hospital, Paterson, N. J.*)

A simple method applicable to samples of the order of 0.0001 to 0.1 Gm. (dry weight basis) was developed for routine clinical use. It is based upon the formation of colored ferric perchlorate derivatives of the hydroxamic acids. The latter are produced when the fatty methyl esters are treated with hydroxylamine in anhydrous media. The reactions involved are quantitative under properly controlled conditions. Each step requires but a few minutes.

A spectrophotometric study of several hydroxamates of biological interest is presented, together with data dealing with normal and pathologic blood lipids, stool lipids in sprue and other idiopathic steatorrheic conditions, pancreatic conditions, and several types of tissue fats. With the use of micro equipment, the method could probably be extended to analysis of elution products from chromatographic or electrophoretic lipid separations.

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Interference of bromide on the Zak "ferric chloride-sulfuric acid" cholesterol method and a means of eliminating this interference. E. W. Rice and Dolores B. Lukasiewicz (*Clinical Laboratory of Presbyterian and Woman's Hospitals, and the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.*)

The method of Zak and associates [*Am. J. Clin. Path.* **24,** 1307 (1954)] has been employed satisfactorily for the routine determination of serum cholesterol. However, a particular "total cholesterol" sample from a patient with acute brominism (serum bromide, 50 mEq./L.) resulted in the formation of a reddish-orange color instead of the typical purple. The "free cholesterol" fraction of this serum had the usual appearance.

Subsequent investigation revealed that the ferric chloride-sulfuric acid color reaction for cholesterol gives erroneously high results in the presence of even traces of bromide. The effect of various quantities of NaBr added to a cholesterol standard is measurable with NaBr concentrations as low as 5 μg. or less per tube—levels at which the color appears a normal purple to the eye. Errors are encountered in the analysis of sera containing as little as 2 mEq./L. of bromide. Although the values for total cholesterol are incorrect, the free cholesterol levels are essentially unaffected. This is because the cholesterol digitonide precipitate is freed of bromide contamination by decanting and washing with acetone.

Iodide gives a color comparable to that of bromide. The Zak color reaction is inhibited by bromate and iodate. Neither bromide nor iodide affects the determination of cholesterol by the Liebermann-Buchard reaction.

The interference of bromide on the Zak cholesterol method can be eliminated simply by shaking the serum briefly with a small quantity of solid silver sulfate prior to extraction with alcohol-acetone mixture.

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Infrared analysis of tissue lipids. H. P. Schwarz, R. Childs, L. Dreisbach, and S. V. Mastrangelo (*Laboratory, Philadelphia General Hospital, Philadelphia, Pa.*)

Infrared analysis of tissue lipids requires (1) availability of methods for quantitative infrared analysis of small samples in the fractional milligram range; (2) establishment of a catalog of spectra of pure compounds isolated from tissue and/or synthesized showing uniqueness of absorption bands which can serve for quantitative analysis of single components or simple mixtures, (3) standardizations based on such pure compounds; and (4) chromatographic separation of lipids extracted from tissues yielding either pure single components or simple mixtures suitable for quantitative infrared analysis.

1. The method for quantitative infrared microanalysis has been established by adaption of the KBr disc technic;

2. Lecithin and cephalin both show a C=O ester vibration at about 5.74 μ which is absent in other phosphatids. At higher wavelength (9–11 μ) the compounds can be distinguished as lecithin shows a strong absorption band at

10.30 μ which is absent in cephalin, and cephalin shows an absorption band at 9.70 μ which is absent in lecithin. Sphingolipids (sphingomyelin and cerebrosides) show amide vibrations at 6.04 μ and 6.40 μ which can be used for analytical purposes.

3. Standardization is based best on spectra of stable synthetic compounds, which should be compared with naturally occurring material whenever possible. It was found, for example, that the extinction coefficient of the C=O band at 5.74 μ of chromatographically purified egg lecithin does not significantly differ from the extinction coefficient of synthetic L-α-dimyristoyl lecithin obtained from Dr. E. Baer. This finding allows use of the synthetic standard for estimation of the naturally occurring compound. Standardization of cephalin was based upon spectroscopy of synthetic L-α-cephalin, standardization of sphingomyelin on material obtained from Dr. G. Schmidt and/or by ourselves through partition chromatography of crude commercial substance.

4. Chromatography with silicic acid columns allows good separation of sphingolipids, cephalin, and lecithin, and probably other lipid constituents of tissues. A chloroform solution of tissue lipids loaded onto the column can be separated into a chloroform fraction containing fatty acids, glycerides, cholesterol and cholesterol esters, and a phosphatid and sphingolipid fraction which is adsorbed on the column and can be eluted with increasing concentrations of methanol in chloroform. This gradient elution technic of adsorption chromatography gives good separation of individual lipids as lecithin, cephalin, and sphingolipids. Infrared analysis of the chromatograms were checked by chemical analysis if possible. Chromatograms of lipids of brain tissue of the rat and skin tissue of the rabbit are demonstrated.

52

Serum glycoproteins and lipoproteins in idiopathic hyperlipemia and idiopathic hypercholesteremia. E. Sohar, Elaine T. Bossak, and D. Adlersberg (*Dept. of Medicine, Tel-Hashomer Government Hospital, Tel Aviv, Israel,* and *The Departments of Medicine and Chemistry, The Mount Sinai Hospital, New York, N. Y.*)

Abnormal deposition of protein-bound carbohydrates has been observed in the arteries in human and experimental atherosclerosis. A correlative study of serum protein-bound carbohydrates ("glycoproteins"), lipoproteins, and proteins seemed profitable in normals and in persons with idiopathic hyperlipemia and idiopathic hypercholesteremia. It is well established that these errors of lipid metabolism carry a predisposition to early and extensive atherosclerosis.

The technic of paper electrophoresis was used with subsequent staining with the periodic acid-Schiff method for glycoproteins. For lipoproteins Oil Red O, and for proteins Amidoblack 10B were used. Idiopathic hyperlipemia (10 patients), characterized by serum lactescence and marked elevation of triglycerides, showed an increase in α_2-glycoprotein to 48.9% of the total stainable carbohydrate. In idiopathic hypercholesteremia the serum was clear and the α_2-glycoprotein (40.5%) did not differ from the controls (41.6%). There was no change in serum α_2-glycoprotein in healthy controls following a standard fat-

loading meal despite elevation of serum triglycerides and increase in the O-fraction of the lipoproteins.

The serum lipoprotein pattern was characteristically changed in both abnormalities of lipid metabolism. Idiopathic hyperlipemia exhibited an elevation of the O (origin)-fraction to 39.9% (control 12.3%) and a relative decrease in α-lipoprotein to 9.2% (control 35.3%). In idiopathic hypercholesteremia there was an increase in β-lipoprotein to 64.8% (control 52.4%), with an increase of the O-fraction to 18.9%. Slight decreases in albumin and increases in β- and γ-globulins were noted in both groups. The alteration in serum glycoproteins and lipoproteins observed in idiopathic hyperlipemia seemed to be, to a certain extent, independent of the serum lipid levels. The observed changes in α_2-glycoprotein and in the O-fraction of the lipoproteins were noted even when, following the use of low-fat diets, the sera showed delactescence and marked reduction of triglycerides toward normal ("masked hyperlipemia").

The importance of these findings for the diagnosis and understanding of these errors of lipid metabolism is discussed.

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The ionographic determination of serum lipoproteins and the fractionation of Sudan Black B. H. J. McDonald and E. W. Bermes, Jr. (*Department of Biochemistry, The Graduate School and the Stritch School of Medicine, Loyola University, Chicago, Ill.*)

The most satisfactory conditions for the ionographic separations of α- and β-serum lipoproteins were found to be: (1) veronal buffer, pH 8.6, ionic strength 0.05; (2) Whatman No. 1 filter paper, 0.5 inch in width; (3) potential gradient 8 volts, migration time, 4 hours, temperature, 4° or 25°. Serum was prestained, prior to application to the paper strips, as follows: 0.1 ml. of a 500 mg./100 ml. solution of Sudan black B (regular or acetylated [*Fed. Proc.* **14,** 733 (1955)]) in 95% ethanol was added slowly, with shaking, to a tube containing 1 ml. of fresh human serum. After 30 minutes the alcohol was removed under reduced pressure, while a stream of air or nitrogen from a thinly drawn capillary was allowed to blow over the surface of the serum to offset frothing and to stir the solution. After the alcohol was removed, the serum was centrifuged to remove excess dye particles. Five λ aliquots of the prestained serum were applied as a thin streak across the paper strips. After a run, the lipoprotein zones appeared as blue bands against a white background. The ionograms were sprayed with a 10% solution of polyvinylpyrrolidone (PVP) to fix the color.

In view of the fact that no alteration in the ratio "total lipoprotein" to "alpha lipoprotein" was observed, it would appear that no extraction of lipid from the lipoprotein complexes occurred, since it is known that lipids are more easily extracted from the β- than from the α-lipoprotein fraction. Since the mobilities of the lipoprotein fractions were unaltered by prestaining, it would appear that they had not been denatured.

In an attempt to obtain a homogeneous and specific lipid stain, Sudan black B was fractionated by column chromatography. Twenty milligrams of the dye were dissolved in 50 ml. of commercial-grade isooctanes and added to the same

solvent in the column, which had been wet-packed with a 4-to-1 mixture of Celite and silicic acid. The solvent level was maintained at a height of 25 cm. above the packing during the run. The flow rate was 70–80 ml./hour. After 96 hours, during which time 4 fractions had been collected, the solvent system was altered so as to contain 3% acetone by volume. This increased the rate of movement of the remaining components. During the next 28 hours 5 more fractions were collected and 1 eluted from the dried column. The colors of the 10 components, in the order in which they came from the column, were: yellow, red, orange, green, blue, greenish blue, blue, blue, blue, and black. Qualitative tests indicated that all fractions possessed some ability to stain lipid material but not serum proteins.

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A new rapid method for estimation of α- and β-lipoprotein cholesterol. J. Claes, A. M. Baerts, and J. V. Joossens (*Medical Departments and Central Laboratory of Clinical Chemistry, St. Raphael, University of Louvain, Belgium*) Abstract to follow.

55

Fat ingestion and serum lipoproteins: studies by starch electrophoresis in normals and in persons with idiopathic hyperlipemia. F. Paronetto, Chun-I Wang, and D. Adlersberg (*Departments of Medicine and Chemistry, Mount Sinai Hospital, New York, N. Y.*)

By starch electrophoresis, idiopathic hyperlipemia was shown to be characterized by marked elevation of both cholesterol and phospholipid content in the α_2-lipoprotein fraction, whereas idiopathic hypercholesteremia exhibited a considerable elevation of these factors in the beta lipoprotein fraction. [*Clin. Res. Proc.* **4**, 91 (1956)].

In continuation of this study 2 patients with idiopathic hyperlipemia were treated with low-fat diets for 1 week and 3 months, respectively. In 1 a marked reduction of serum lactescence occurred with a decrease of total lipids from 3000 to 2100 mg./100 ml. These changes were associated with a decrease in the cholesterol from 55 to 4.6% and phospholipid from 63.5 to 12.9% in the α_2-lipoprotein. There was a marked increase of these factors in the β-lipoprotein (cholesterol from 40.5 to 92.6%, phospholipid from 27.8 to 80.5%) without any significant changes in the α_1 lipoprotein. The second patient showed delactescence of serum, a drop of total lipids from 2300 to 1220 mg./100 ml. and changes in the lipoprotein fractions similar to those observed in the first patient. Thus the use of a low-fat diet in idiopathic hyperlipemia affected serum total lipids and triglycerides, lowered the concentration of both cholesterol and phospholipid in the α_2-lipoproteins, and raised the concentration of these factors in the β-lipoprotein.

The effect of a fat-loading test (2 Gm. of butter fat/kg.) was studied in 2 normal controls. At the height of alimentary lipemia there was a decided increase in cholesterol (3.4 to 13.5%) and in phospholipid (1.0 to 16.5%) in the α_2-lipoprotein fraction with a concomitant decrease of these factors in α_1-lipoprotein, from 38 to 21.3% for cholesterol and from 60 to 43.5% for phospholipid, without significant changes in β-lipoprotein. The alterations of α_2-lipoprotein in normals

after fat intake resembled somewhat those seen in persons with idiopathic hyperlipemia on fat-free diets.

The elevation of cholesterol and phospholipid in the α_2-lipoprotein fraction in idiopathic hyperlipemia exceeds several times that seen after ingestion of fat in the normal and decreases drastically on fat-free diets.

SESSION 10. Protein-Bound Iodine; Mucoproteins

56

Protein-bound iodine levels in resorcinol-induced hypoplasia of the thyroid. J. H. Webster (*Ann Arbor, Mich.*) (Abstract to follow)

57

The ultramicro determination of total and protein-bound iodine. M. C. Sanz, T. Brechbühler, and I. J. Green. (*Hôpital Cantonal, Geneva, Switzerland*)

The method presented is based on the one described by Barker. After ashing the sample with alkali at 600° the formed iodide is photometrically determined by its catalytic effect on the reduction of ceric sulphate by arsenic acid.

For serum (total iodine or PBI) and urine a volume of 50 µl. is used, for saliva 5 µl., corresponding to quantities of 1–12 µg. of iodine. The precision of the determination is approximately ±1%.

The Beckman DU spectrophotometer is used with 10 mm. micro quartz cuvets at a wavelength of 317 mµ corresponding to the maximum of the absorption of ceric sulphate. At this wavelength all other substances present in the solution do not show any appreciable absorption.

The special equipment used is described and the precautions which only can guarantee reproducible results are discussed.

The results of precision-, reproducibility-, and recovery-controls as well as some normal and pathological values are presented. The normal values for human serum are: total iodine 3.77–5.97 µg./100 ml. with a mean value of 5.22 µg./100 ml.; PBI 3.50–5.60 µg./100 ml., mean value 4.89 µg./100 ml.

The advantages of the described method lie not only in the use of very small sample volumes, permitting determinations on newborn children and on animals, but also in an appreciable gain of time and simplicity.

58

Use of continuous and strip paper electrophoresis technics for the study of serum glycoproteins. M. R. Shetlar, C. Cahill, G. Stidworthy, and Clara L. Shetlar (*Research Laboratory, VA Hospital and the Department of Biochemistry, University of Oklahoma School of Medicine, Oklahoma City, Okla.*)

Using a continuous paper electrophoresis technic, fractionations of a number of sera of patients with rheumatoid arthritis and cancer have been carried out and compared with similar studies of normal and pregnant subjects. These fractions have been analyzed for glycoprotein and protein content. Parallel studies of proteins and glycoproteins were conducted by conventional paper strip electrophoresis technics, utilizing the same sera. Comparison of the two technics will be discussed.

By use of these technics the serum fraction or fractions responsible for the

elevated glycoproteins of arthritis, cancer, and pregnancy may be detected. Possible use of these technics as aids in evaluating patients will be considered.

59

Glyco- and Mucoproteins in the Cerebrospinal Fluid: Determinative methods and application. Elizabeth Roboz, Anna-Mae Luft, R. R. Apostol, and W. C. Hess (*The Departments of Biological Chemistry and Neurology, Georgetown University School of Medicine, Washington, D. C.*)

Certain neurologic diseases affect the chemical composition of the cerebrospinal fluid. The protein-bound carbohydrates have been studied, using recently devised methods for the determination of total and γ-globulin-bound carbohydrates. Analysis of a large number of fluids from patients with various neurologic diseases showed that the greatest increase of protein-bound carbohydrate occurs in brain tumor.

Methods have been developed for the determination of mucoprotein, α-protein, and total glycoprotein in the cerebrospinal fluid. In the fluid the average ratio of the carbohydrate moiety of the mucoprotein to the total bound carbohydrate was 38.6 ± 4.7, and in the sera 12.3 ± 2.1. When fluids and bloods of the same patients with advanced neurologic disease were examined, it was found that the ratio in the fluid was 40.2 ± 3.5 and in the sera 18.5 ± 6.2.

The ratio of carbohydrate to tyrosine of the mucoprotein in the fluid was 4.7 ± 1.5, whereas in sera it was 3.4 ± 0.98. The ratio of carbohydrate to tyrosine of the glycoprotein in the fluid was 1.1 ± 0.28 and in sera 0.54 ± 0.13.

The carbohydrate content of the α-glycoprotein has been determined in the fluid: the ratio to the total carbohydrate was 39.0 ± 15.0. The muco- and α-glycoprotein, according to these studies, account for about 78 per cent of the total protein-bound carbohydrates.

60

Study of staining techniques reported selective for protein-bound carbohydrates and lipids on electropherograms of serum. W. Q. Wolfson and B. Pennwarden (*The Laboratory and Medical Services, U. S. Army Hospital, and the Office of the Regimental Surgeon, 18th Infantry Regiment, Fort Riley, Kans.*)

After separation on Munktell 20-B in *p*H 8.6, 0.06M Michaelis buffer, serum protein paper-electropherograms stained with bromphenol blue have been compared with duplicate strips stained (1) for lipid with Macdonald's acetylated Sudan Black B prerun technic; (2) for lipid by post-run technic, and (3) for carbohydrate with crystal violet, toluidine blue, and Schiff leukofuchsin, the latter three being compared after no oxidation, or bromine, or periodate oxidation.

Toluidine blue metachromasia increased with bromine or periodate, but the final pattern with toluidine or crystal violet exactly paralleled the bromphenol blue pattern in locus and relative intensity of bands. Since isolation studies show a virtual absence of carbohydrate in albumin, little in γ-globulin, and highest concentrations in α-globulin, these two stains are predominantly staining other components of the electropherogram. Recolorized Schiff leukofuchsin is highly variable, and results depend on complex wetting factors and other varia-

bles, such as differential elution rates of protein-bound leukofuchsin and recolorized fuchsin during washing. If all of these factors are carefully controlled, the reported predominant staining of α-globulin does not appear and results are still inexplicably variable. These findings and an absolute lack of parallelism with toluidine metachromasia make Schiff staining highly suspect as to specificity.

In contrast to these problems in staining of protein-bound carbohydrate, prerun staining of lipoprotein with acetylated Sudan Black B appears to be an elegant improvement in technic suitable for use on starch or paper. Combined with bromphenol blue, the two stains make valuable prerun tracers and do not detectably change the protein pattern obtained.

61

A comparison of the salivary lysozyme content of caries-active and caries-inactive individuals. Susan C. J. Gouge and G. W. Burnett (*Dental Division, Walter Reed Army Institute of Research, Washington, D. C.*)

Early studies of lysozyme indicated that it functioned in the human defense mechanism by lysing or inhibiting certain microorganisms. Variations in concentrations of lysozyme in the body were found to be an index of pathology. An accurate method for determining lysozyme concentration in body fluids has been devised by Lobstein and Fogelson. Using this method, the lysozyme content of the saliva of sixteen persons was determined on five or more successive days. Contrary to previous findings no appreciable difference was noted between the caries-active individuals (average mean value: 26.11 μg. lysozyme/ml. of saliva) and the caries-inactive individuals (average mean value: 33.23 μg./ml.). The individual with the highest mean concentration (48.2 μg./ml.) and the one with the lowest mean concentration (9.84 μg./ml.) were both caries-active. Significantly, there was a day-to-day variation in the lysozyme content of the saliva of an individual.

62

The colorimetric determination of ketone bodies in blood and urine by means of vanillin in alkaline medium. V. E. Levine and M. Taterka (*Creighton University School of Medicine, Omaha, Nebr.*)

Vanillin in alkaline medium reacts with a large number of organic compounds with a carbonyl group. Because of the wide reactivity of the reagent it cannot be used for any specific carbonyl compound unless it is first isolated. Acetone has a low boiling point, 56.5°. It can therefore be readily separated by aeration or distillation from other components in a biologic fluid or tissue.

We have found that of all the carbonyl compounds investigated acetone yields the maximal color, with an 0.8 % vanillin in 10N sodium hydroxide, 2 gamma being easily detected. The vanillin-alkali reagent has advantages over the salicylaldehyde-alkali reagent of Benedict and Behre for ketone bodies. Vanillin is a white crystalline compound that can be weighed accurately. It can be purified by crystallization from water. It is more stable than salicylaldehyde, which is a liquid, insoluble in water, deteriorating very rapidly even when kept

in a brown container. Salicylaldehyde must be purified by distillation before use.

The method requires the removal of acetone by distillation of urine or protein-free blood. In acidified solutions it can also determine diacetic acid. The acetone, or acetone plus diacetic acid, is distilled into a sodium bisulfite solution. An aliquot part of the distillate (1 ml.) is treated with 1 ml. of 0.8% aqueous vanillin and 2 ml. of 10N sodium hydroxide. The reaction mixture is placed in a water bath at 60° for 15 minutes. The mixture is diluted to 6 ml. and read in a Beckman spectrophotometer at 420 μ.

SESSION 11. Symposium on Enzymes

O-1

Diabetogenic action of xanthurenic acid and its relationship to carbohydrate metabolism. Y. Kotake (*Biochemistry Department, Wakayama Medical College, Wakayama, Japan*)

When a fairly large amount of both sodium butyrate and tryptophan was administered at one time to albino rats, diabetic symptoms (hyperglycemia, glycosuria, etc.) were observed, due to the production of xanthurenic acid, an abnormal metabolite of tryptophan. Our experiments led to the conclusion that these diabetic symptoms were closely related to diabetes in man.

After biochemical examination of the diabetogenic action of xanthurenic acid, the main cause of diabetic symptoms was concluded to be the inhibition of hexose-phosphorylation; in animals, the inorganic phosphate in blood and liver increased, while the amount of phosphocreatine decreased; furthermore, the amount of fructose-6-phosphate and fructose-1-6-phosphate in the liver also decreased. The activity of yeast and animal hexokinase was inhibited by xanthurenic acid. On this occasion anthranilic acid, 4-hydroxy-8-methoxyquinoline-2-carboxylic acid, ethereal sulfate of xanthurenic acid, and kynurenic acid not only acted as inhibitory agents to the diabetogenic action of xanthurenic acid, but confirmed the finding that these agents acted as antagonists to xanthurenic acid in regard to hexokinase activity.

O-2

Chronic diabetic symptoms caused by xanthurenic acid, an abnormal metabolite of tryptophan. Y. Kotake (*Biochemistry Department, Wakayama Medical College, Wakayama, Japan*)

When a fairly large amount of tryptophan was administered at once to albino rats together with sodium butyrate, urinary excretion of xanthurenic acid, an abnormal metabolite of tryptophan, was observed and diabetic symptoms such as hyperglycemia, glycosuria, etc., were observed in these rats.

Many experiments were carried out with crystallized xanthurenic acid and it was demonstrated that xanthurenic acid was diabetogenic. Next, experiments on the chronic diabetogenic action of xanthurenic acid were carried out: albino rats were fed on a high-fat-and-casein and vitamin-B_6-deficient diet for a long period; in order to promote the accumulation of xanthurenic acid in the body, small amounts of tryptophan were daily added to the diet at certain periods. We finally succeeded in obtaining a relatively severe and chronic diabetic condi-

tion. In this case, remarkable pathologic changes were observed in the β-cells of the Langerhans islets of the pancreas. On the other hand, we succeeded in detecting urinary excretion of xanthurenic acid in diabetic patients. From these facts it was concluded that the diabetic symptoms caused by xanthurenic acid in albino rats are distinctly inhibited by administration of vitamine B_6, anthranilic acid, methionine, and 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid.

P

Significance of some vitamins of the B-complex in clinical chemistry. N. Siliprandi (*Institute of Biological Chemistry, University of Camerino, Italy*)

The approach to the problem of the metabolic significance and of the action mechanism of the B vitamins has been recently made possible by the observations that all known coenzymes are derivatives of B group vitamins.

The nutritional requirement of these vitamins (thiamine, riboflavin, nicotinamide, vitamin B_6 and pantothenic acid) can be explained on the basis of their coenzyme function. In all cases the coenzymes appear to be the only metabolically active form for these particular vitamins.

On the basis of this biochemical statement a systematic work in clinical chemistry has been started to study in pathologic conditions the transformations of B-complex vitamins into their corresponding coenzymes. The possible relationships between metabolic disorders and the impaired efficiency of the coenzymes has also been considered.

The clinical syndromes in animals or man following a deficiency of one or more vitamins are known, but this is not the case of the underlying metabolic alterations. An increasing light on the mechanism by which the clinical symptoms are caused is thrown, however, by the insight into the metabolic functions of coenzymes.

Furthermore, metabolic disorders attributable to the inefficiency of one or some coenzymes may not always be explained by an inadequate supply of the vitamin, but are sometimes due to a decreased power of the organism to transform them into the corresponding coenzyme.

Work has been started on this line and significant results have been obtained in experimental and human diabetes and in some liver diseases. An altered metabolism of the coenzymes deriving from thiamine (cocarboxylase) and from riboflavin (FMN and FAD) has been evident in such conditions.

Q

Significance of the products of the citric-acid cycle in clinical chemistry. Jo Nordmann and R. Nordmann (*Laboratoire de Biochimie, Clinique Chirurgicale de la Salpêtrière, Paris, France*)

The existence of rather simple technics for citric acid and α-keto-acid determinations in body fluids has allowed numerous observations which are discussed. The authors have described a chromatographic technic which gives a whole picture of the citric-acid cycle: all the stable acids of the cycle, except isocitric, have thus been detected in urine, most of them quantitatively; the quantitative

relations between them are pointed out. This technic has been applied to animals and to various clinical states.

The alterations of citric-cycle acids in urine during liver injury are compared to the known disturbances of blood citric and α-keto-acids. The alterations of these acids in renal insufficiency, diabetes, and other diseases are also discussed.

The dynamic exploration of the citric-acid cycle may be carried out by studying the variations of the acids of the cycle in blood and urine induced by perfusion of various metabolites. Previous results (which deal essentially with normal animals) are reported and compared to those achieved through chromatography. The major abnormalities found especially in tubular nephritis and in hepatic injuries are discussed.

Finally the clinical interest of citric-cycle enzymes, inhibitors, and relations between citrate and calcium are pointed out.

R

Significance of enzymes in clinical chemistry. E. J. King (*Postgraduate Medical School, London, England*)

Enzymes are used in modern medicine for diagnosis and prognosis, and, to a limited extent, for certain therapeutic purposes. Digestive enzymes in the stomach, small intestine, and pancreas, and in the stool have a relation to gastric and pancreatic function and to absorption. Uropepsinogen, as well as pepsin, has a relation to anemia and gastric secretion. Serum lipase is raised in acute pancreatitis. Amylase, both in urine and in plasma, is similarly raised and may also be of diagnostic value in mumps. Alkaline phosphatase is a test for the differential diagnosis of jaundice. It is markedly elevated in generalized bone disease. Plasma acetylcholinesterase is decreased in cases of poisoning by modern insecticides like parathion; it is also used as a test of hepatic function. Serum acid phosphatase, aldolase, and phosphohexose-isomerase are elevated in prostatic and breast cancer, and are a good index of the growth of metastatic bone tumors and also of the effect of treatment. Transaminase is raised in myocardial infarction. Several proteolytic enzymes of the plasma may have a diagnostic use. Other proteolytic enzymes have been successfully employed for the debridement of dead and necrotic tissue, and for the liquefaction of viscous accumulations of mucus and pus. Trypsin and papain are used to activate red cells to render them agglutinable by various forms of incomplete antibody.

SESSION 12. Electrophoresis

63

Electrophoretic patterns of serum proteins after major operations. J. Sternberg, G. Préfontaine, G. Labelle, and G. Boulet (*Institute of Microbiology and Hygiene, University of Montreal, and St. Joseph Hospital, Rosemount, Montreal, Canada*)

The electrophoretic pattern of serum proteins has been examined in patients undergoing various operations (pneumonectomy, lobectomy, thyroidectomy, nephrectomy, one hypophysectomy). The blood samples were taken immediately

before operation and at 12, 24, and 48 hours and 5, 7, 10, and 14 days after operation. Further controls were done every month until 3 months after operation. Various other tests were carried on simultaneously, chiefly the sedimentation rate, the C reactive protein, and polysaccharide determination.

In the case of hypophysectomy, when samples were taken during the 9 hours operation, the electrophoretic pattern remained remarkably stable, despite the different pattern of the 3500 ml. transfused blood. In all other cases examined immediately after operation (12 hours), no significant change was noted. The α-globulins increased slightly on the second and third day after operation, but the most significant change occurred much later, during the second week. The α_2-globulin, and to a lesser extent the α_1-globulin, increased to an average value of 150% of the preoperative values. The noted increase was significantly parallel to an increase of the sedimentation rate and of the polysaccharides. The C reactive protein did not show the same correlation as that of the ESR.

The most consistent "second-week α-globulinic crisis" was noted in patients with lung operations, whereas the other types of operations occasionally showed the same phenomenon. Of 2 nephrectomies, 1 was accompanied by an increase of the sedimentation rate as well as of the α-globulin level, while the other case did not vary at all during 15 days after operation.

The increase of the α-globulins and of the protein-bound carbohydrates might be taken as a readaptive reaction of the body, similar to the increase of tissular polysaccharides noted during the reparation period of a wound.

Thus, an increase of the ESR noted in a patient during the second week after an operation must be interpreted as a normal reaction of the body and not always as a sign of a superimposed inflammation.

64

Electrophoretic studies of body fluids in a case of hydatid mole. J. Sternberg, G. J. Strean, and M. M. Gelfand (*University of Montreal, and Jewish General Hospital, Montreal, Canada*)

The paper electrophoretic separation of chorionic gonadotrophins, tried in urine of normal and pregnant women, has shown that the CG, with a *p*H of 4.65, has a migration zone situated between the two α-globulins. A characteristic feature of this zone is its high polysaccharide content, giving a positive fuchsin-periodic acid staining reaction.

In one case of hydatid mole the blood and the urine were collected before operation as well as every day for several days after operation. The liquid content of the removed hydatid mole was extracted; also a parovarian cyst found during the operation was punctured and its content kept for examination.

The electrophoretic pattern of serum proteins showed an increase of the α_2-globulins in the second week after operation. This phenomenon cannot be correlated to any hormonal disturbance but rather to a readaptive reaction of serum proteins after postoperative shock; indeed, it has been noted by others after many operations.

The hydatid mole extract, titrating approximately 2,000,000 units CG/ml., has an electrophoretic pattern with a high peak in the interalpha region. An

extraction of the above zone and a further bioassay showed that the chief amount of CG was concentrated in this region. In opposition to the urinary gonadotrophins, the polysaccharide staining reaction of the interalpha zone was completely negative.

The electrophoretic pattern of the parovarian cystic fluid, titrating 700 units CG/ml., was composed of a high albumin fraction and a few low globulin fractions. The albumin fraction was completely devoid of hormonal activity.

The urinary extract had an electrophoretic pattern similar to that found in the urine of pregnant women; the interalpha zone gave a positive carbohydrate fuchsin staining reaction. This fact might be interpreted as a difference in the structure of the hormone extracted from the molar vesicles and that extracted from the urine.

65

Serum protein changes in thermal trauma. R. E. Deadrick, J. L. Morico, E. R. Lozano, F. Tausig, R. P. Hummel, and G. F. Lanchantin (*Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas*)

Previous studies have shown that in severe thermal trauma, marked alterations in plasma proteins, lipoproteins, and glycoproteins occur. Many of these investigations have been confirmed to animal experimentation in order that the degree and area of burn could be controlled and thus permit qualitative and quantitative evaluation of the effects elicited. The present study was undertaken in an attempt to assess the changes in the various serum protein complexes in thermal trauma of the human and to attempt to relate these changes to the severity and area of burn as well as therapeutic treatment. At the time of the preparation of this abstract, 20 adult patients with minimal to lethal burns were studied. Because of an efficient air evacuation system many of these patients arrived and were studied in this unit within the first 24 hours post burn.

The technics employed were those of moving boundary electrophoresis at *p*H 8.6 and *p*H 4.5, zone electrophoresis at *p*H 8.6, and analysis of serum cholesterol, phospholipid, and total glycoprotein.

Our data indicates that in the immediate postburn period there is a significant reduction in the concentration of γ-globulin accompanying a marked hypoalbuminemia in patients having a burn index greater than 4 (B.I. = % area 3° burn + ¼% area 2° burn). At about the seventh to tenth postburn day α-globulin increases to levels above normal, but albumin remains at a reduced level until skin grafting. $Alpha_2$-globulin is elevated during this period and returns to normal upon skin coverage of the burned area. $Alpha_1$- and β-globulin remain within normal concentration limits, although cholesterol is markedly reduced. These changes as well as changes in the glycoprotein level are discussed.

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Quantitative study of dye-protein relationship by scanning and elution methods in paper electrophoresis. J. V. Joossens and J. Claes (*Medical Departments and Central Laboratory of Clinical Chemistry, St. Raphael, University of Louvain, Belgium*) Abstract to follow.

67

Paper electrophoresis of serum and urinary proteins in the diagnosis of myelomatosis. J. A. Owen and W. D. Rider (*Department of Clinical Chemistry, Clinical Laboratory, Royal Infirmary, Edinburgh, Scotland*)

The value of paper electrophoresis in the diagnosis of myelomatosis has been critically assessed.

Serum and urinary proteins from 40 patients with myelomatosis have been examined. In 33 patients the serum protein patterns clearly contained an abnormal zone of protein, the distribution of these zones being: α_2-type, 1; β-type, 5; and γ-type, 26. In 7 patients the serum proteins showed only minor changes. However, 6 of these patients had proteinuria, the urinary protein in the 5 urines examined consisting almost entirely of β-globulin. Thus, 38 out of 40 cases of myelomatosis had characteristic protein patterns in the serum or urine. However, 4 other patients, believed not to have myelomatosis but with no definite diagnosis, were found also to have serum protein patterns of the "myeloma" type.

The incidence of other findings was as follows: plasmocytosis in biopsy specimens, 93 per cent (of patients with myelomatosis); anemia (Hb. <10 gm./100 ml.), 80 per cent; E.S.R. (>70 mm./hr.), 76 per cent; radiologic bone changes, 75 per cent; proteinuria, 68 per cent; hyperproteinemia (total protein >8 Gm./100 ml.), 55 per cent; hyperglobulinemia (globulin >5 Gm./100 ml.), 49 per cent; Bence Jones proteinuria, 28 per cent. On this reckoning, biopsy appears to be the most valuable single test. However, none of these findings, including plasmocytosis of bone marrow, is specific to myelomatosis.

These results indicate that paper electrophoresis of serum and urinary proteins provides evidence confirming, or reinforcing, the diagnosis of myelomatosis in a very high proportion of cases. In practice, however, the correct diagnosis is very frequently made without the help of this procedure.

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The effect of a high oxygen atmosphere on the serum proteins of newborn animals. D. H. Higginbotham, Miriam Reiner, and A. Patz (*Department of Chemistry, District of Columbia General Hospital, and the Chemo-Medical Institute and Department of Ophthalmology, Georgetown University, Washington, D. C.*)

Newborn young from three different species—dogs, cats and rats—were placed in a high-oxygen atmosphere for the first days of life. The high-oxygen atmosphere produced an eye lesion similar to retrolental fibroplasia in human premature infants. Electrophoretic studies of the sera of normal newborn kittens and newborn oxygen-treated kittens showed that the high-oxygen atmosphere lowered the relative per cent of γ-globulin and increased the relative per cent of α_1-globulin. This lowering of γ-globulin (natural immunity factor) agreed with the clinical finding that newborn kittens in a high-oxygen atmosphere were very susceptible to colds and respiratory ailments. The other serum proteins—albumin, α_2-globulin, and the combined β-globulins—showed little differences.

Electrophoretic studies of the sera of normal newborn puppies and newborn oxygen-treated puppies showed that the β-globulin was increased and the other protein fractions decreased in the oxygen-treated animal. There was an apparent lowering of the γ-globulin fraction associated with an increase in age.

Electrophoretic studies of the sera of normal newborn rats compared with newborn rats kept in a high-oxygen atmosphere showed an increase in the albumin fraction and a corresponding decrease in the γ-globulin fraction.

69

The effect of caloric restriction on the metabolism of N^{15}-labeled glycine in pregnant women. C. Alper and J. Seitchik (*Hahnemann Medical College, Philadelphia, Pa.*)

Earlier studies of these authors demonstrated that calorically adequate low-protein, low-purine diets (36 Gm.) induced an enhanced rate of conversion of glycine-N^{15}, to uric acid-N^{15} in pregnant women. These data were interpreted to indicate an attempt by the body to maintain the tissue nucleoprotein mass in the face of relative nitrogen deficit. Because of the known protein-sparing effect of carbohydrate, pregnant women who received low caloric diets (1500 calories) of varying content (40 and 120 Gm.) were studied. In this manner the effect of caloric restriction on the conversion of glycine-N^{15} to uric acid-N^{15} could be determined, presumably the effect of caloric restriction on the utilization of glycine for nucleoprotein synthesis. Pregnant women, 28–32 weeks gravid, were prepared for glycine administration by dietary priming of 9–14 days' duration during which time the miscible pool of uric acid was determined. Glycine (N^{15} content, 60–64 atoms per cent excess) was administered orally in a dose of approximately 80–100 mg./kg. of body weight. For the succeeding 8 days the daily total nitrogen, urea and ammonia (as a single fraction), and uric acid excretion was determined as well as the isotope concentration of these individual constituents. Although the uric acid pool and the uric acid portion of total excreted nitrogen was similar in all patients, the dietary restriction of calories results in a reduced rate of turnover of glycine-N^{15} to uric acid-N^{15}. Apparently, the ability of the pregnant female to maintain the nucleoprotein mass in the face of relative nitrogen deficit requires adequate caloric intake.

70

Ionophoresis of the buffer salts during zone and bidimesional electrophoresis. H. Peeters (*Laboratory, St. Jans Hospital, Brugge, Belgium*)

A study of the ionophoresis of the sodium and veronal ion during uni- and bidimensional electrophoresis was carried out. A typical salt pattern builds up over the substrate. Shape and localization of the richer and poorer zones are of fundamental importance because they modify the ionic strength and conductivity of the substrate.

The origin of this pattern was studied. Potassium ions were systematically substituted for sodium ions in different portions of the buffer and by this means

the building-up of the salt pattern could be followed. The image for both salts is not quite identical but the building-up follows the same trends.

Conductivity of eluates of the substrate was measured and led to the conclusion of different field strength in different parts of the substrate. These local changes in ionic strength and conductivity are greatly responsible for the normal distribution of protein fractions. When the salt shifts over the substrate are still greater they cause changes in *p*H which are a common cause of bad results.

These experimental facts brought us to the study of a simple means—e.g., rapid control of *p*H over the entire bidimensional field—of controlling the conditions of a run. Applying this and other technics a rapid evaluation of new buffer conditions is rapidly obtained and many useless trials are avoided.

71

Paper-electrophoretic analysis of gastric juice in health and disease. G. B. J. Glass, Loukia Stephanson, and Marilyn Rich (*Gastroenterology Research Laboratory, New York Medical College, Flower and Fifth Avenue Hospitals, New York, N. Y.*)

Attempts to quantitate high molecular components of gastric juice by chemical fractionation, chromatography, or Tiselius electrophoresis did not result in satisfactory resolution of various mucoproteins, enzymes, and other nondialyzable substances present in the gastric juice. After over 2 years of trials with various concentration methods, apparatus, buffers, papers, and stains the authors have successfully adapted paper-electrophoretic technic to the routine analysis of gastric juice in health and disease.

Forty to 60 λ of 2% solution of dialyzed and freeze-dried gastric juice is dissolved in borate buffer (*p*H 9.0 and ionic strength 0.12) and applied to Whatman #1 filter paper strips in hanging paper-strip Spinco cell. After 4½–5½ hours of electrophoresis at a relatively high milliamperage the strips are dried in the oven and stained for routine use with amidoblack 10 B, and for investigational purposes also with PAS and light green FS stains, and scanned, traced, and integrated in the servo-type integrating scanner Analytrol provided with cam B_{12} and 575 mμ filter.

Excellent resolution was obtained with amidoblack stain into 7–8 and more well-duplicated and reproducible peaks. On the anodic side of the partition were identified pepsin peak (P), which stains with amido-black and FS, and four mucoprotein peaks (M_{1-4}) which take up all three stains. On the cathodic side 3–5 yet unidentified peaks were obtained (X, Y, Z) stained with amidoblack only, which may correspond to some alkaline enzymes, gastrin, and organic nondialyzable bases.

Typical paper-electrophoretic patterns are presented of normal gastric juices under fasting conditions and following histamine and insulin stimulation, and their changes during various phases of gastric secretion. Also pathologic paper-electropherograms of gastric juices in diseases of the stomach will be shown and their clinical significance for recognition of hypersecretory stomach, gastritis, and gastric atrophy are discussed.

72

Chromatography in microbiologic studies of infection in burns. J. A. Rivera (*Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas*)

These studies include observations on the chemistry of pigments produced in vivo and in vitro by certain microorganisms isolated from infected wounds and from the blood of patients with septicemia at a burn center. The biosynthesis of the pigments, their variation in color, and their chemical changes are correlated with oxidation-reduction potentials and metabolic factors. Changes in the quality and quantity of pigment production after antibiotic and other drug treatments are described. Spectrophotometric observations on eluted pigments are briefly considered. Methods of extraction and concentration of pigments in preparation for chromatography, as well as the choice of solvents, are described. Separation and identification of pigments by a new chromatographic procedure is discussed at length and chromatograms are compared with each other.

SESSION 13. Lipid Metabolism

73

Plasma lipids and coagulation of blood. E. Sohar, M. C. Rosenthal, and D. Adlersberg (*Dept. of Medicine, Tel-Hashomer Government Hospital, Tel-Aviv, Israel,* and *Departments of Medicine, Hematology, and Chemistry, the Mount Sinai Hospital, New York, N. Y.*

Coagulation studies were performed on 7 controls, 6 persons with idiopathic hyperlipemia, and 5 persons with idiopathic hypercholesteremia, before and after a standard fat meal. In none of the subjects was there an alteration of the standard clotting time, three-tube silicone clotting time, standard prothrombin time, serum prothrombin activity, thrombin generation and thromboplastin generation.

There was, however, depending upon their triglycerides content, a distinct difference in the behavior of various plasmas to the action of "incomplete" thromboplastin ("Stypven"). "Stypven" prothrombin time showed definite shortening in 4 of 5 healthy controls following fat ingestion. In the 1 subject who showed no shortening, the expected rise in serum triglycerides (neutral fat) failed to occur. In the persons with idiopathic hyperlipemia "Stypven" prothrombin times were abnormally shortened prior to fat ingestion and were only slightly influenced following the fat meal. In the persons with idiopathic hypercholesteremia the "Stypven" prothrombin time was as in the controls. Chemical and electrophoretic studies revealed shortening of the "Stypven" prothrombin time to be correlated with an increase in serum triglycerides. Centrifugation of hyperlipemic plasma into two fractions, fat rich and fat poor, also revealed a correlation between fat content and shortening of the "Stypven" prothrombin time. No change in erythrocytic fragility, osmotic or mechanical, was revealed following ingestion of food. Intact platelets, and even more so disrupted platelets, exerted an effect similar to that of triglycerides, in that they shortened the "Stypven" prothrombin time.

The presence in the plasma of a thromboplastin potentiator (triglycerides) either postprandially in the normal, or, perhaps more important, constantly in the hyperlipemic subject, may result in increased coagulability and thrombosis when thromboplastic material is liberated into the blood stream.

74

A fat-tolerance test. D. E. Beischer and S. Born (*U. S. Naval School of Aviation Medicine, Pensacola, Fla.*)

The method uses the ingestion of a large amount of fat in the form of one quart of ice cream (20% fat) to test the metabolic control of fat transport. Venous blood samples drawn before the ingestion of the fat load and at certain intervals (conveniently 1, 4, and 24 hours) thereafter were analyzed for cholesterol, and a study of the lipoproteins by the ultracentrifuge method introduced by Gofman was made. While the changes of the cholesterol concentration of the blood serum were in all cases insignificant, some of the lipoprotein fractions and the arteriogenic indices (A.I.) showed a characteristic behavior. In some individuals, the normal plasma picture is rapidly restored after the ingestion of fat. Other subjects, mainly with high A.I. from the start, show a sluggish removal of the chylomicrons. The A.I. may increase up to 50 per cent from its normal value, mainly caused by increases of the S_f 100 to 400 and 20 to 100 fractions. The high values of these fractions may persist for one or two days. The S_f 12 to 20 fraction is not significantly changed and changes in the S_f 0 to 12 fraction may be positive or negative.

The fat-tolerance test reflects in a more subtle way metabolic deficiencies of fat breakdown than the mere statement of abnormal lipoprotein concentrations. This test may find clinical interest since a better localization of the block in the clearing process may suggest specific means of treatment of this metabolic deficiency.

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Metabolic fecal fat excretion in tropical sprue. C. F. Asenjo, R. Rodríguez Molina, and Marta Cancio (*Department of Biochemistry and Nutrition, School of Medicine, University of Puerto Rico and San Patricio V.A. Hospital, San Juan, P. R.*)

The steatorrhea observed in tropical sprue generally has been ascribed to a poor absorption of exogenous fat. It is the object of this communication to describe a series of fat-balance experiments carried out in order to determine whether, in sprue patients, the so-called metabolic fat excretion (fecal fat of endogenous, tissue, and bacterial origin) is of the same order of magnitude as in normal subjects. Sprue patients were given a steatorrhea test diet supplying 90–95 Gm. of fat per day for a period of 8 days. Patients showing abnormal fat absorption (a daily fecal fat excretion of more than 6 Gm.) were then given a diet supplying less than 10 Gm. of fat per day for a period of at least 12 days. Samples of the food consumed and the feces excreted were collected daily throughout the trials. These samples were analyzed for total fat by the van de Kamer method [*J.B.C.* **177**, 347, (1949)]. The results obtained so far indicate that the daily fecal fat

excretion of sprue patients on a low-fat diet is of the same order of magnitude as that of normal subjects.

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Fat synthesis by intestinal bacteria. A. C. Frazer and H. G. Sammons (*Department of Pharmacology, Medical School, University of Birmingham, England*)

Fat synthesis has been demonstrated in vitro with a strain of *S. faecalis* recovered from the stools of patients with steatorrhea. The yield of fat was increased by control of acid formation and the addition of folic acid. Under suitable conditions, 30–50 Gm. of fat was formed from 1 L. of a solution of 1% glucose and 2% peptone in 5 days. Most of this fat was synthesized on the third and fourth days. The fat consisted of glyceride esters of long-chain fatty acids.

Studies with labeled fat in the patient from whom the first fat synthesizing organism was obtained indicated normal fat absorption, in spite of an average daily fecal fat level of 15–20 Gm. The fecal fat was rapidly reduced to 4–5 Gm a day by the administration of antibacterial agents. Seven earlier patients with sprue and continuing steatorrhea responded similarly to antibacterial therapy. The general significance of these findings is discussed.

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A macromolecular complex in human gallbladder bile as the stabilizing factor for cholesterol. J. C. M. Verschure (*Medical Clinics of the State University, Utrecht, The Netherlands*)

In gallbladder bile we found—by means of paper electrophoresis—a complex compound that was stainable with a protein stain (azocarmine) and a lipid stain (Sudan black B) and which contained the bilirubin and the largest part of the desoxycholic acids and cholesterol. At the same time Isaksson (thesis, Lund 1954) isolated this compound with chemical extraction methods. It is soluble in chloroform *and* in water, and is able to yield in water about one tenth of its weight of cholesterol. This compound may thus be of great importance in keeping cholesterol in solution in the bile. With electrophoretic and ultracentrifugal studies we proved that the molecular weight of this bile complex is about 26,000 and that it contained a maximum of 7 molecules of cholesterol per molecule, and as a mean 3.4 mol. Of bilirubin the mean content was 0.57 molecules; the maximum content was found at 9.4 mol. per molecule of the complex. Thus the capacity to keep bilirubin in solution is normally less involved than in the case of cholesterol. With ultracentrifugal experiments, macromolecules analogous to that of gallbladder bile could be demonstrated in fistula bile, in much lower concentrations. The strong nucleus of the complex, consisting of about 8 molecules of lecithin with about 40 molecules of desoxycholic acids, could be synthesized. It was comparable with the complex obtained by Isaksson with chemical extraction, but showed various properties different from the native complex obtained with electrophoresis; for instance, it did not stain with azocarmine, was unable to bind bilirubin under various conditions, and did not show the characteristic yellow fluorescence of the native complex. The conditions under

which this complex is formed, its protein component, and its role in the prevention of bone stone formation are discussed.

78

Serum neuraminic acid levels in the disease state. A. Saifer and Shirley Gerstenfeld (*Biochemistry Department, Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, N. Y.*)

Tay-Sachs' disease is an illness of early infancy characterized by arrested development, amaurosis, progressive paresis, and death. Pathologically, progress of the disease is accompanied by marked accumulation of cerebroside-like lipids designated by Klenk as "gangliosides." It was later demonstrated that neuraminic acid (NA) was a major component of the "gangliosides" of Tay-Sach's disease and closely related disorders. Elevated tissue NA levels are apparently specific for such lipoidoses. Recently quantitative determinations with the colorimetric diphenylamine reaction has shown this amino-sugar (NA) to be present in serum. In this study, starch block-electrophoresis of both normal and Tay-Sachs' sera was employed to locate the protein fractions which contained NA. Results obtained showed an approximately equal distribution of total neuraminic acid (TNA) between the albumin and the globulin fractions of normal sera but a significant increase in the globulin neuraminic acid (GNA) as compared to albumin in Tay-Sachs' disease. Based on these findings, proteins from 37 normal and 116 pathologic sera were separated into a soluble albumin fraction and an insoluble total globulin fraction using salt fractionation. Neuraminic acid levels were then determined on the whole serum (TNA) and on the globulin fraction (GNA) in each case. The nonspecific TNA (diphenylamine reaction) is elevated in many inflammatory and malignant diseases, e.g., rheumatoid arthritis, cancer, etc. The more specific GNA/TNA ratio was about normal in only about 4 per cent of 106 pathologic cases studied, except for Tay-Sachs' and related diseases in which it was elevated in 100 per cent of the 10 cases (or 85 per cent of 43 individual samples) tested. The methods are discussed.

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The influence of uremia on the apparent cholesterol level in serum. A. M. Baerts, J. Claes, and J. V. Joossens (*Medical Departments and Central Laboratory of Clinical Chemistry, St. Raphael, University of Louvain, Belgium*) Abstract to follow.

80

A simplified procedure for studying fat absorption and utilization using I^{131}-labelled triolein or oleic acid. D. A. Turner (*Surgical Research Metabolic Laboratory, Georgetown University Hospital, Washington, D. C.*)

Stanley and Thannhauser (1949) reported on the use of I^{131}-labeled olive oil in studies of fat absorption and metabolism. I^{131}-lipid activity of serum was calculated by subtraction of water-soluble I^{131} activity from total serum activity. The water-soluble I^{131} was separated from lipid-I^{131} by coprecipitation of the serum lipid with serum protein using the Somogyi reagents. Free inorganic I^{131} is also precipitated with serum protein by this procedure and represents an important source of error under certain conditions.

Ruffin and his group (1955) and others have measured the total blood or fecal activity after a meal of I^{131}-triolein. No attempt was made to measure actual lipid activity.

A simplified and accurate procedure for the direct estimation of I^{131}-lipid activity in blood or tissue after the oral or intravenous administration of I^{131}-triolein or I^{131}-oleic acid is presented.

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The investigation of pancreatic insufficiency using I^{131}-triolein and oleic acid. D. A. Turner, O. Parker, R. J. Coffey, and B. J. Duffy, Jr. (*Surgical Research Metabolic Laboratory, Georgetown University Hospital, Washington, D. C.*)

I^{131}-triolein and I^{131}-oleic acid tolerance tests have been used to differentiate the steatorrhea of pancreatic insufficiency from that due to other causes.

The steatorrhea of pancreatic disease may be due either to a decrease or an absence of lipolytic activity in the intestinal lumen. In these subjects the absorption of I^{131}-triolein is markedly diminished. This has been demonstrated by a flat I^{131}-lipid blood tolerance curve and an elevated I^{131}-lipid fecal content.

A flat tolerance curve with low blood I^{131} activity and increased fecal I^{131} activity has also been found in patients with sprue, Crohn's disease, dumping syndrome, and after massive resection. I^{131}-triolein and I^{131}-oleic acid tolerance tests have been employed to differentiate steatorrhea of pancreatic origin from that caused by mucosal or other abnormalities.

In pancreatic disease triolein absorption is decreased and oleic acid absorption is normal. In mucosal or mechanical abnormalities there is impaired absorption of both the triglyceride and its constituent fatty acid.

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Ca^{45} measurements in the presence of I^{131}-triolein in studies of steatorrhea. T. Wheeler, B. J. Duffy, Jr., and D. A. Turner (*Surgical Research Metabolic Laboratory, Georgetown University Hospital, Washington, D. C.*)

The effect of steatorrhea on calcium absorption and utilization is being investigated using I^{131}-triolein and Ca^{45}. A method for the direct measurement of Ca^{45} and I^{131}-triolein in the same blood sample is presented.

The role of steatorrhea as a possible factor in osteoporosis and osteomalacia is discussed.

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Effect of soybean phosphatides on antibody production. C. A. Slanetz (*Columbia University, New York, N. Y.*) Abstract to follow.

SESSION 14. Inorganic Blood Constituents

84

A comparative study of calcium metabolism between the lordotic and normal guppy (*Lebistes reticulatis*) H. L. Rosenthal (*Division of Biochemistry, Department of Pathology, Rochester General Hospital, Rochester, N. Y.*)

Elucidation of the mechanism for calcification and bone metabolism has been hampered by the lack of suitable experimental animals in which specific mecha-

nisms can be experimentally induced or altered. The discovery of a naturally occurring mutation in the common guppy (*Lebistes reticulatis*) which appears as a markedly lordotic spine and an increased concentration of body calcium may prove to be important in animal experimentation.

Analysis of vertebral bone from the lordotic strain indicates a significantly greater calcium concentration but the phosphorus concentration of lordotic and normal bone is the same, and the increased bone calcium concentration is associated with a greater spine–body weight ratio. The water, fat, protein, calcium, and phosphorus concentrations of muscle tissue are the same for both strains of fish. Bone salt crystal structure from x-ray diffraction studies indicate a typical dahlite structure for the lordotic and normal strains, but the lordotic spines show a more linear orientation of bone salt crystals than the normal.

The rate of uptake of calcium^{45} by the fish from water in which they swim is significantly lower for the lordotic than for the normal guppy. However, the rate of incorporation of calcium^{45} into verterbral bone is greater than normal. The turnover rate of calcium^{45} by vertebral bone for both strains is similar and approximates a biological half life of over a year.

These studies suggest that hereditary lordosis in these animals is associated with an increased deposition of calcium in bone salts due to a genetic alteration in the mechanism of bone formation.

85

A study of the colorimetric end point in EDTA calcium titrations on human serum. Leonore H. Koehler (*St. Luke's Hospital, Bethlehem, Pa.*)

In using the Klett colorimeter for the photometric end point in the direct ethylenediamine titration of calcium in serum, the procedure of Fales [*J. Biol Chem.* **204,** 577 (1953)] and of Horner (*J. Lab. Clin. Med.* **45,** 951 (1955)] had to be modified. The working volumes were made smaller, using micro size tubes, to give maximal color increments necessary with the #62 filter, and to facilitate mixing by hand. To 0.5 ml. of serum in a micro Klett tube are added 1.5 ml. of water, 0.5 ml. of 1.5N sodium hydroxide, and 1 ml. of ammonium purpurate indicator (prepared according to Horner, and not more than 2 weeks in refrigerator). Without delay, titration is performed using a solution containing 1 part of stock ethylenediamine tetraacetate, disodium salt (3.6 Gm./L.) to 1 part indicator solution. After an initial addition of 0.15 ml., a plot, as recommended by Fales, is traced of color increments on adding 0.02 or 0.025 ml., until readings remain the same; total titration is usually less than 0.3 ml. Reproducibility is good, the end point very clear, and there is agreement with the Clark-Collip method if phosphate is not over 6 mg./100 ml. A calcium chloride standard is used to calibrate.

A precipitation procedure for serum is modeled on that of Horner (for urine, etc.): in a 15 ml. centrifuge tube, 1 ml. of serum plus 1.5 ml. of water and 0.5 ml. of sodium tungstate are stirred well (slim glass rod) with 1 ml. of morpholine reagent. After 1 hour and centrifugation at 3000 rpm for 10 min., titration is performed as above; 2 ml. of the supernatant, equivalent to 0.5 ml. serum, are first neutralized with NaOH, 10% (amount about 0.2 ml., gauged by titrating

dummy mixture with methyl orange indicator). Higher results than on serum directly may be due to liberation of calcium ion from complex. This effect, also the effect of *p*H, is being studied.

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In vitro calcification of rat and rabbit aortae. B. Eichel, S. W. Morgenstern, and A. E. Sobel (*Department of Biochemistry, Jewish Hospital of Brooklyn, Brooklyn, N. Y.*)

Rat and rabbit aortas were incubated at 37° in inorganic calcifying solution containing 15 mg./100 ml. Ca, 6 mg/100 ml. PO_4, KCl, NaCl and CO_2-bicarbonate buffer. Before placement in calcifying solution, these tissues did not yield a silver stain. After 20 hours incubation, as measured by silver stain, mineralization was evident in the media and intima of rat and rabbit aortas. The mineralization became more intensive with longer periods of incubation. Parallel studies of metachromasia, developed with toluidine blue O, of comparable aorta sections showed that the metachromatic intensity of the media and intima decreased with time as the silver stain intensified. Chemical analyses showed a Ca/PO_4 ratio for the aortas of about 2.0, the calcium being from 2 to 8% of the dry weight. Heating of rabbit aorta for 10 minutes in H_2O at 60° and 90°, respectively, did not inactivate the calcifying mechanism. Rat and rabbit aortas placed in calcifying solutions containing glycyl-glycine buffer mineralized readily in the presence or absence of $NaHCO_3$.

Histopathologic examination of mineralized rat aorta indicates that the mineralization resembles the medial calcification found in Mönckeberg's disease without the accompanying cell necrosis. With respect to the latter, the appearance of the aortas more closely resembles that which is seen in the early stages of experimental hypervitaminosis D.

These findings indicate that the aorta possesses a system capable of mineralization in vitro and may represent clues to the mineralization of aorta in vivo.

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The role of chondroitin sulfate in the calcifying mechanism. A. E. Sobel and M. Burger (*Department of Biochemistry, Jewish Hospital of Brooklyn, Brooklyn, N. Y.*)

Rachitic bones, demineralized with EDTA, after treatment with chondroitin sulfate will remineralize in the epiphysis and diaphysis but not in the metaphysis. The metaphysis will mineralize following treatment with chondroitin sulfate if the bone is treated with calcium chloride. The noncalcifying cartilage will not mineralize. Thus, there is evidence of two related calcifying mechanisms in bone.

Demineralized teeth, treated with chondroitin sulfate and calcium chloride, will remineralize in calcifying solutions.

Two types of collagen chondroitin sulfate mixutres were prepared, that from the rat tail collagen will mineralize without calcium chloride treatment but that from the Achilles tendon of steer requires calcium chloride treatment. These appear to correspond to the two systems found in bone.

A tentative explanation of the calcifying mechanism is presented based on collagen chondroitin sulfate complexes.

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The effect of bone bank preservation on the calcifying mechanism. L. S. Lavine, M. Burger, and A. E. Sobel. (*Department of Surgery, Division of Orthopedic Surgery, State University of New York College of Medicine at New York City, and the Department of Biochemistry, Jewish Hospital of Brooklyn, Brooklyn, N. Y.*)

To test the influence of bone bank preservation methods on the calcifying mechanism, calcification in vitro of rachitic rat cartilage was employed. It was found that the present methods of bone storage inactivate the calcifying mechanism. This inactivation was reversible for some methods of bone preservation.

The methods studied of preserving bone were: (1) deep freeze, (2) aqueous merthiolate, (3) acetone, and (4) the boiled-bone technic. All of these methods are utilized for storing bone for orthopedic use.

When preserved bone was placed in a calcifying medium, new mineralization was zero compared to controls of fresh bone which showed excellent calcification. However, when preserved bone was treated with a calcium chloride solution, the calcifying mechanism was reactivated; most easily in deep-freeze bone, next in merthiolate-treated bone, to a lesser degree in acetone-treated bone, and least in boiled bone. The calcium chloride treatment prior to freezing prevented inactivation. The deep-freeze bone gave almost no metachromasy unless calcium ions were added to the toluidine blue. The significance of this is discussed.

The results of these studies indicate that the value of stored bone may be enhanced by proper chemical treatment; this fact has important clinical significance. Further animal investigations will be conducted on this problem.

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Calcification of implanted rachitic bone slices. M. Burger, L. S. Lavine, and A. E. Sobel. (*Department of Biochemistry, Jewish Hospital of Brooklyn, Brooklyn, N. Y., and the Department of Surgery, Division of Orthopedic Surgery, State University of New York College of Medicine at New York City.*)

Rachitic bone slices were placed under the skin of young rats fed rachitogenic and normal diets. Calcification of the epiphyseal hypertrophic cartilage, with typical line test, was observed in bone sections implanted in rats on a normal diet but not in bone sections implanted in rats on a rachitogenic diet. Studies of calcification of rachitic bones in vitro, in an inorganic medium, indicated that this difference is probably due to the higher product of calcium and phosphate ions (Ca $\times$ P) in the body fluids of animals on a normal diet as compared to those on a rachitogenic diet.

Employing this technic, frozen bones treated first with $CaCl_2$, as well as demineralized bones treated with chondroitin sulfate, showed better mineralization of the hypertrophic proliferating bone cartilage than the untreated frozen bones or demineralized bones. The implications of these findings are discussed.

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The urinary amino acids in relation to calculus disease. Mary G. McGeown (*Department of Medicine, Queen's University of Belfast, Belfast, Northern Ireland*) Abstract to follow.

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The diagnosis of hyperparathyroidism. Mary G. McGeown (*Department of Medicine, Queen's University of Belfast, Belfast, Northern Ireland*) Abstract to follow.

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Serum and cerebrospinal fluid magnesium levels in conditions of altered electrolyte metabolism. A. A. Henly and R. A. Saunders (*Biochemistry Laboratory, Little Bromwich General Hospital, Birmingham, England*) Abstract to follow.

93

The concentration of calcium in the serum of normal adults. M. H. Power, Ruth Toogood, and Lucille Adamson (*Section of Biochemistry, Mayo Clinic and Mayo Foundation, Rochester, Minn.*)

The normal range of serum calcium is often stated to be 9.0 to 11.0 mg. per 100 ml., although some investigators have reported values as low as 8.0 and as high as 11.8. Our own experience has suggested that the range of normal, under the conditions of our analyses, is considerably narrower than these limits would indicate. In order to provide further orientation, we have determined calcium in the serum of 107 individuals who were considered to be "normal" on the basis of physical examination. The group was about equally divided between men and women. Venous blood was drawn in the morning before breakfast and the serum was separated within an hour or two thereafter. Analyses were based on direct precipitation of calcium as oxalate, and titration of the washed precipitate with permanganate. Single determinations by each of two analysts were done, the procedures differing as to size of sample (2 ml. and 1 ml.), time of precipitation, technic of washing, and technic of titration. The observations ranged in magnitude from 9.19 to 10.61 in the first series, and from 9.17 to 10.65 in the second; the distributions were approximately normal, and the mean values and standard deviations were 9.77 ± 0.31 and 9.81 ± 0.30, respectively.

94

Comparison of venous and capillary blood electrolyte analysis. Harry G. Anrode, and W. W. McCrory (*The Children's Hospital of Philadelphia and the Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pa.*)

In the past few years there has been an increasing awarenss of the need and advantage of applying ultramicrochemical technics to clinical chemistry. These methods are especially desirable in connection with pediatric services where broad biochemical studies have heretofore often been limited by the difficulty of obtaining sufficient blood. It remains to be shown that a feasible application

of ultramicrotechnics to routine laboratory procedures gives values which compare favorably with those of the standard macro-technics.

It has been shown previously by other investigators, and also in this laboratory, that there is no significant difference between results obtained on the same serum by either macro or ultramicrotechnics. It is of interest to know whether significant differences are found to exist between serum obtained by venipuncture or heel prick (capillary blood) in sick infants and children.

The concentrations of CO_2, chloride, sodium, and potassium in venous and capillary blood obtained simultaneously and treated in identical manner were determined. CO_2 analyses were done in a modified Kopp-Natelson apparatus. Chlorides were determined by the electrometric titration described by Seligson. Sodium and potassium analyses were done on a Baird flame photometer adapted to use General Electric photocells.

It is shown that there is no difference between the sodium and potassium of venous and capillary serum. The CO_2 of capillary serum is approximately 1 mM/L. lower than that of venous serum, while the chloride of capillary serum is approximately 1 mEq./L. higher. Results of 25 such simultaneous determinations are presented and analyzed.

95

Estimation of serum sodium levels from bicarbonate and chloride determinations. K. M. Dubowski (*Division of Clinical Chemistry and Toxicology, Iowa Methodist Hospital and Raymond Blank Memorial Hospital for Children, Des Moines, Iowa*)

Estimation of serum sodium levels by calculation from serum bicarbonate and chloride determination values is useful whenever flame photometric sodium analyses are not readily available, and for the independent approximate verification of serum sodium levels obtained by flame photometry.

The dozen or so formulas suggested for this calculation attest to the practical importance of the problem in clinical chemistry, but unfortunately show considerable variation in factors and therefore in the correctness of the computed sodium value. To be clinically useful, the estimation formula should be applicable in a variety of normal and abnormal electrolyte states and should yield acceptably useful values in unselected patients. Not all existing formulas meet these criteria.

The availability of well over 1000 simultaneously performed serum sodium, bicarbonate, and chloride level determination results in the routine work of our laboratories afforded an opportunity to test the concordance between experimental serum sodium levels obtained by flame photometry and the estimated sodium values calculated from the bicarbonate and chloride levels, using the several major formulas, in a large series of both adult and pediatric patients with a variety of clinical disease states.

Such testing of the various suggested formulas together with statistical treatment of the data validly demonstrates the reliability of each formula and permits the derivation of a new and effective formula for sodium estimation from bicarbonate and chloride level values.

96

Use of PVP as AgCl dispersing agent in the turbidimetric determination of blood serum chlorides. R. S. Wayne and I. J. Greenblatt (*Messinger Research Laboratory, Beth-El Hospital, Brooklyn, N. Y.*)

A Somogyi filtrate of blood serum is prepared to which is added a standard $AgNO_3$ solution. The $AgNO_3$ is in aqueous 5% PVP. After 10 minutes of incubation at 88°, the solution reaches stability and maintains a constant turbidity for about 3 hours. This has been compared to the method of Schales, and runs parallel to the Schales method ($\pm$ 1.3 mEq./L.).

SESSION 15. Various Blood and Urine Constituents

97

The diagnostic value of barbiturate analyses. I. Sunshine (*Cuyahoga County Coroner's Office, Cleveland, Ohio*)

The differential diagnosis of a comatose patient should include barbiturate intoxication. The incidence of this type of intoxication will vary with the community and its customs. Clinical chemists can be very helpful in these situations by performing a qualitative and quantitative barbiturate analysis on a sample of the patient's blood. Data will be presented to indicate that both types of barbiturate determinations are essential and significant. Tolerance to these drugs can be acquired through prolonged use. Chemical data will be presented to illustrate this. Should death supervene, barbiturate analyses of biologic material is essential to supplement the pathologic findings. Both a toxicologic and pathologic examination are essential to document a diagnosis of death due to barbiturate intoxication. Data in some typical postmortem material are presented. Formalin-fixed tissues can be useful if fresh tissue samples are not available. Barbiturates are detectable in both tissues and the formalin solution as long as 18 months after death.

98

The determination of ammonia in blood, plasma and erythrocytes. D. Seligson (*The Division of Biochemistry of the Graduate Hospital and the Department of Biochemistry of the Graduate School of Medicine of the University of Pennsylvania, Philadelphia, Pa.*)

Ammonia is isolated from blood and other biologic fluids by using a modification of the microdiffusion system of Seligson and Seligson [*J. Lab. and Clin. Med.* **38,** 324 (1951)] which is described. The blood is alkalinized with potassium carbonate and bicarbonate to keep the reaction mixture between *p*H 11 to 11.5. Under the circumstances of the analysis, ammonia diffuses from the blood-reaction mixture at the same rate as from a water-reaction mixture, thereby requiring no correction factors. Furthermore, no significant hydrolysis of blood constituents occurs to contribute artifacts. Use of the potassium carbonate-bicarbonate buffer for alkalinization appears to have important advantages over potassium carbonate in that hydrolysis is diminished, diffusion rates from blood and water are the same, and recoveries of added ammonia are better. The diffused ammonia is easily collected and measured photometrically in this diffusion system.

The ammonia found in blood by this method appears to be preformed ammonium ions. When blood is exposed to air and allowed to stand, ammonia develops at the constant rate of about 0.003 μg. NH_3—N per ml. per min.; an amount which is negligible under the described conditions. In 29 patients free of liver disease, the mean ammonia nitrogen found in whole blood, plasma, and erythrocytes was 1.37, 0.78, and 2.20 μg. per ml. respectively. The erythrocyte ammonia was found to be 2.8 times that found in plasma.

99

A preliminary report on the partition of organic acids in body fluids on silica gel columns. S. Meites (*Children's Hospital, Columbus, Ohio*)

The method of Isherwood (1946) has been applied to the determination of the carboxylic acids, exclusive of α-amino acids, in urine and other body fluids of man, in an effort to establish the normal composition in health and variation in disease. Details of the method are presented. The position of elution of some 60 known acids will be demonstrated. All acids, with the exception of the α-keto-acids, are eluted quantitatively. Preliminary results indicate that 8 fractions are obtained from urine. The initial fraction is a complex mixture of aromatic compounds. The remaining fractions, though not pure, consist of acetic, formic, lactic, citric, and two unknowns. The aromatic acid fraction constitutes the greatest fraction in the urine of normal individuals. While the method does not permit recovery of all organic acids in urine, results indicate that the daily organic acid output of premature infants is diminished when compared to older children or adults on a body surface basis.

100

Bovine platelets, serotonin, and the retraction of human plasma clots. R. L. Fenichel (*Department of Physiology and Pharmacology, Wayne University, Detroit, Mich.*)

A study of the phenomenon of clot retraction employing a dialyzed human plasma assay procedure has indicated that serotonin (5-hydroxytryptamine) is a clot-retraction principle. A prepared bovine platelet dialysate has also been shown to possess clot retraction activity when this assay procedure is employed.

Adsorption of citrated human plasma with barium carbonate removes a non-dialyzable clot-retraction principle from this plasma. Addition of specially prepared barium carbonate eluates to barium-carbonate-adsorbed plasma restores its clot retraction activity.

Glycerol inhibits clot retraction.

Human citrated plasma can be made deficient in both nondialyzable and dialyzable clot retraction principles by barium-carbonate adsorption and dialysis against a large volume of physiologic saline. These clot-retraction activities can be replaced by addition of serotonin and human barium-carbonate eluate or by addition of bovine-platelet dialysate and human barium-carbonate eluate. The *p*H for this series of experiments is maintained at 6.8 so that it is not a factor contributing to these results. Serotonin and bovine-platelet dialysate therefore both function with human barium-carbonate eluate to promote retraction of plasma clots when an adsorbed and dialyzed human-plasma assay system is employed.

101

The role of a contaminant in thrombin in human plasmin assay systems. M. Siegel and E. E. Cliffton (*Sloan-Kettering Institute for Cancer Research and Cornell University Medical College, New York, N. Y.*) Abstract to follow.

102

The problem of specificity in the chemical determination of urinary catecholamines. W. B. Mason, J. Salvatore, and J. L. Potter (*Department of Biochemistry and Atomic Energy Project, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.*)

In an attempt to improve the specificity of the urinary catecholamine determination, a procedure has been developed which includes: (a) preliminary collection of catecholamines on aluminum hydroxide precipitated at *p*H 7.4; (b) removal of urinary pigments by *n*-butyl alcohol extraction of the precipitate dissolved in hydrochloric acid; (c) reprecipitation of aluminum hydroxide at *p*H 7.4; (d) digestion of the precipitate with sodium citrate, ammonium hydroxide and ethylene diamine at *p*H 10.5–11.5; (e) extraction of the digest with *n*-butyl alcohol; (f) separation of extracted products by paper chromatography using an *n*-butyl alcohol-water-ammonia system; and (g) quantitation by spectrofluorimetry. Epinephrine and norepinephrine are easily differentiated, both by the wavelength of their fluorescence and by their R_f values. Applied to normal and pathologic human urines, this procedure yields a number of fluorescent products. These results are described and discussed briefly.

103

Measurement of glutathione turnover rates. J. W. Goldzieher and P. K. Besch (*Southwest Foundation for Research & Education, San Antonio, Texas*)

The only technic specific for measurement of GSH—glyoxalase—is too cumbersome for routine use and unsuited for determination of specific activity and turnover rates in our problems in glutathione metabolism. Exploration of other possibilities led to study and modification of the classical cadmium-precipitate method. This proved superior in specificity and for handling large numbers of samples, but was inferior in sensitivity and precision to our previously published amperometric technic [Goldzieher, Rawls, and Goldzieher, *J. B. C.* **203**, 519 (1953)]. The two methods complement each other.

A series of 25 recovery experiments were run. In tissue homogenates, oxidation to S—S and other reactions caused apparent loss of SH. The mean recovery values for testicular and liver tissue were 58.4%, ± S.D. 18.8 and 57.6%, ± S.D. 9.9 respectively.

Radioactive cadmium glutathionate in 2% sulfosalicylic acid was counted both by standard planchet technics and by liquid scintillation in a system containing water, ethanol, and toluene in the ratios 0.5:33:66. 2,5-Diphenyloxazole (3 Gm./L.) and 1,4-di-(2-[5-diphenylozazole]) benzene (0.03 Gm./L.) were used as phosphors and at this concentration gave the maximum count per unit time. The samples were counted in a Packard Tri-Carb Liquid Scintillation spectrometer.

104

The colorimetric determination of micro quantities of thiocyanate in biological fluids. M. Feldstein and N. C. Klendshoj (*Division of Toxicology, University of Buffalo, Buffalo, N. Y.*)

Thiocyanate is determined in blood or urine filtrates by reaction with chlorine and a barbiturate-pyridine reagent which forms a red color in the presence of thiocyanate ion. Cyanide, which interferes, is removed by aeration from acid solution. The reaction will detect 0.1 μg. of thiocyanate. The color is relatively stable and is measured in a spectrophotometer at 580 mμ.

105

Quantitative determination of *p*-aminosalicylic acid and isonicotinic acid hydrazide and its principal hydrazine-yielding metabolites in blood. J. R. Maher, J. M. Whitney, J. S. Chambers, D. J. Stanonis (*Research and Development Service, Fitzsimons Army Hospital, Denver 8, Colo.*)

Four methods for the quantitative determination of isonicotinic acid hydrazide have been carefully investigated. The Kelly-Poet method and a modification thereof have been shown to measure total INH; i.e., free isonicotinic acid hydrazide and its conjugated metabolites. It has not been demonstrated microbiologically that the conjugated metabolites possess bactericidal activity. The picryl chloride method of Hunter was investigated and found lacking in sensitivity requisite for clinical application. The vanillin method reported by Deeb and Vitagliano was investigated and its specificity for free INH in the presence of its conjugated metabolites was confirmed. However, the protein-free filtrate they described was observed to contain certain interfering chromogens. Goldman's investigation of the absorption spectrum of free INH in various *p*H environments was confirmed. The possibility of exploiting ultraviolet absorption as a quantitative method for isonicotinic acid hydrazide and its metabolic conjugates in peripheral blood was explored. A highly erratic absorption background in drug-free sera rendered this method untenable.

A method is described which quantitatively determines free isonicotinic acid hydrazide in the presence of its conjugated metabolites and *p*-aminosalicylic acid in peripheral blood. The method also permits the quantitative determination of total isonicotinic acid hydrazide (free plus conjugated metabolites) and of *p*-aminosalicylic acid in one specimen of blood.

The necessity of combining chemotherapeutic agents for the successful treatment of tuberculosis leads the authors to believe that this method will find general application in the routine clinical chemistry laboratory as well as in the research laboratory.

106

The nature of the Ehrlich benzaldehyde reaction on indole compounds. *I. Paperchromatographic, spectrophotometric, colorimetric and spectrophotometric measurements.* H. Sprince, G. R. Rowley, Dorothy Jameson, F. E. Manson, I. F. Bennett, and F. C. Dohan. ***II. Factors affecting the nature of the reaction.*** G. R. Rowly, H. Sprince, Dorothy Jameson, F. E. Manson, I. S. Bennett and F. C. Dohan. Abstract to follow.

107

Some quantitative aspects of odor control with quaternary ammonium compounds. N. C. Molnar (*Molnar Laboratories, New York, N. Y.*)

The odor-controlling property of those quaternary ammonium compounds which have high bactericidal property has been demonstrated. This property is based upon the fact that bacteria produce enzymes essential to the putrefactive breakdown causing malodors. Examples of this are given by showing the use of odorless quaternary ammonium compounds of low toxicity for keeping chemical toilets on airplanes odorless, by preserving urine specimens from putrid decomposition, in their use in underarm deodorants, etc. However, this author has accumulated evidence that some quaternaries, in themselves odorless, will neutralize the preformed odors caused by putrefaction or otherwise. An explanation is offered for this action based upon quantitative measurements. It is noteworthy that those quaternaries which have bromides as anions are more active odor neutralizers than those that have chlorides. It appears that this is due to the faster and more complete reactivity of bromides. A large number of malodorous substances are anionic in nature, such as sulfides, sulfites, butyrates, valerianates. Even substances, such as dimercaptopropanol, thiocresol, thiophenol, having a rancid skunklike odor react with quaternary bromides such as cetyl trimethyl ammonium bromide forming rather stable large molecular weight substances of extremely low vapor pressure and thus nonodiferousness. The laboratory data to be presented uses for end point of odor neutralization a dependence with only an olfactory observation but also chemical indicators to show the absence of odor producing vapors; for example, in the case of hydrogen sulfide, lead acetate test paper.

A brief résumé will be given of the state of the art as far as the relations between chemical constitution and odors are concerned and present methods of odor detection and measurements are concerned.

SESSION 16. Symposium on Proteins

S

An evaluation of trends in electrophoresis instrumentation. A. Henley (*National Instrument Laboratories, Riverdale, Md.*)

The scope of the subject concerns the principles of current methods employed in studying the size, shape, and electrochemical properties of protein molecules and related substances of biological interest (hormones, enzymes, conjugated proteins, nucleic acids, and nucleoproteins).

Such methods are interrelated with the following parameters: size measurements by means of equilibrium ultracentrifuge alone or by combination of sedimentation velocity and free diffusion data; independent information derived from viscosity, light-scattering, and flow birefringence measurements; usefulness and limitations of individual technics and correlation with electron-optical and x-ray diffraction data; various methods available for shape measurements; effect of electrical charges on molecules and comparison with recent work on polyelectrolytes; determination of electrophoretic mobility and, hence, zeta

potentials by microscopic and moving boundary methods of electrophoresis; usefulness and limitations of zone (paper) electrophoresis.

The more than 50 different electrophoresis units now commercially available are surveyed as examples of all the electron-optical systems so far developed and some future directions of electrophoresis theory are indicated. This wide variety of instruments used in electrophoresis requires examination in the light of two criteria: first, the extent to which the apparatus meets the requirements of the individual research and clinical laboratory; second, whether the instrument lends itself to standardization in this complex field of research, both with regard to procedures and to the evaluation of results.

T

Mucoprotein estimations in clinical chemistry. N. F. Maclagan (*Department of Chemical Pathology, Westminster Medical School, London, S.W.1., England*)

The possible clinical value of mucoprotein estimations first arose out of work on the polarography of cancer sera by Brdicka, when his results were shown to Winzler and his colleagues to depend upon a mucoprotein constituent. Since then, a number of methods of estimation of serum mucoprotein have been elaborated, and these have been applied to a wide range of clinical states by Greenspan, Shetlar, and other workers. More recently, attention has been directed to the urinary mucoproteins by the work of Tamm and Horsfall, and methods for the estimation of a urinary mucoprotein fraction have been elaborated at Westminster Medical School. A strong corollation between a serum and urine mucoprotein concentration has been demonstrated, suggesting that the latter may originate in part from the former.

In general, serum mucoprotein estimations are preferred to those on the urine for technical reasons. The results obtained in cancer, inflammations, and in liver and collagen diseases will be reviewed. The relationship between the mucoproteins and the flocculation tests and liver function will be discussed, and possible disturbances of urine mucoproteins in cases of renal calculi will also be considered.

U

Mucoproteins in clinical chemistry. Z. Stary (*Institute of Biochemistry, Istanbul University, Istanbul, Turkey*)

Various mucoproteins containing relatively large groups of bound carbohydrate have been isolated from blood serum by different methods. Smaller groups of bound carbohydrate are present in almost every fraction of the serum proteins and in fibrinogen. The total amount of protein-bound carbohydrate is about twice as high as that of free glucose. These prosthetic carbohydrate groups are not directly connected with the metabolism of glucose and glycogen. Their main constituents are mannose, galactose, and hexosamine, smaller amounts of neuraminic or sialic acid, and (in some fractions only) hexuronic and sulfuric acid radicals are also present. Contrarily to the rapidly changing glucose level, the level of protein-bound polysaccharides in serum is relatively constant and does not change in a significant degree after insulin or adrenalin administration nor

after carbohydrate intake. Low in the newborn infant, it reaches 160–180 mg/100 ml. in normal adults and increases slowly with age. No significant sexual differences in the level of protein-bound carbohydrate and no changes during the menstrual cycle have been observed. A great increase occurs in the last months of normal pregnancy; higher levels are observed in toxic pregnancy. Many other pathologic conditions can also cause increases in the serum polysaccharide content: Hyperpolysaccharidemias have been observed in animals after heavy blood losses, scurvy, and alloxan diabetes and in humans in diabetes mellitus. Hyperpolysaccharidemias also occur in acute and chronic infectious diseases; these are partly due to the formation of carbohydrate containing antibodies electrophoretically migrating with the γ-globulin-fraction, partly to an unspecific effect of general stress, which raises mainly the carbohydrate content of the α-globulin fraction. Many other factors causing general stress (injections of turpentine, burns, etc.) produce hyperpolysaccharidemias in animals. Likewise high polysaccharide levels have been observed in humans in consequence of allergic reactions, rheumatism, gout, lupus erythematosus, etc.). Hyperpolysaccharidemias of this kind can be reversed, at least partly, by administration of ACTH or cortisone. Malignant tumor growth causes high hyperpolysaccharidemias. Some of the serum polysaccharides form probably particles of linear shape; hyperpolysaccharidemias due to an increase of this sort of particles are accompanied by an increase of ESR, while other forms of hyperpolysaccharidemia (e.g., in diabetes or typhus abdominalis) do not cause an increase of the ESR. The main site of polysaccharide production seems to be the liver; other fractions of serum polysaccharides may originate from damaged or rapidly regenerating tissues. In phosphorus-intoxicated rabbits the increase in serum polysaccharides after bleeding was relatively small when compared to rabbits with undamaged liver, and in humans heavy damage of liver parenchyma (e.g., in cirrhosis) is often accompanied by low polysaccharide levels. Serum polysaccharides seem to be able to migrate into damaged tissues and some of the amyloid and hyaline deposits in damaged tissues may originate directly or indirectly from chronically increased plasma polysaccharides. Protein-bound polysaccharides are also present in cerebrospinal fluid; increases have been observed in cases of brain tumor and multiple sclerosis. The relatively high amounts of polysaccharides present in exudates are partly of plasmatic origin, while other parts are probably due to the mucus secretion of the serosa endothelium. That mucoproteins are physiologically excreted by the kidneys is evident from the occurrence of gonadotrophic hormones in urine; nevertheless, most of the mucoproteins present in urine are produced by mucus-secreting cells of the urine conveying system. A mucoprotein fraction isolated from normal urine is characterized by a specific reactivity with various forms of viruses. Important increases of urine mucoproteins have been observed in cases of calculous disease.

V

Significance of lipoproteins in clinical chemistry. J. C. M. Verschure (*Medical Clinics of the State University, Utrecht, The Netherlands*)

The scope of this review is mainly given by the possibilities of the ordinary well-equipped hospital laboratory, and the demands of the clinic. For the

moment this means in view of technical possibilities: salt or alcohol precipitation methods, clearing factor estimation, several "lipoprotein flocculation tests" and especially lipid diagrams obtained by paper electrophoresis. The rich new literature on these subjects is summarized and discussed from a critical standpoint, trying to show the limitations and possibilities for the clinical chemical laboratory. The preliminary clinical findings of the methods mentioned are given. Around this nucleus of data that lie within the field of clinical chemistry, those other biochemical data are arranged that may open up a wider outlook upon the significance of the lipoproteins in medicine.

SESSION 17. Hormones and Vitamins

108

Determination of plasma levels of 17-hydroxy-corticosterone and corticosterone. J. McLaughlin, Jr., and I. Gray (*Department of Biochemistry, Walter Reed Army Institute of Research, Washington, D. C.*)

The Sweat method for the fluorimetric analysis of plasma concentrations of corticosterone and 17-hydroxy-corticosterone has been modified and used in a study of normal humans and rats. Samples of plasma (1 to 10 ml.) are shaken for 3 minutes with ten times their volume of chloroform. After removal of the chloroform the samples were chromatographed through columns containing silica gel. By use of various mixtures of ethanol and chloroform three fractions are eluted from the columns. After removal of the solvent the samples are taken up in a sulfuric acid and ethanol mixture for development of fluorescence. Together with the plasma samples, three other columns are also run: one column to measure the column blank; one column with a known standard of 17-hydroxy-corticosterone (Compound F); and, one column with a known standard of corticosterone (Compound B). With the data obtained from the various columns it is then possible to set up simultaneous equations to calculate the amount of Compound B and Compound F present in the plasma samples.

Data are presented to show specificity, advantages, limitations, recoveries, and ways of increasing the sensitivity of the procedure. Values are presented for plasma levels of corticosterone and hydrocortisone (17-hydroxy-corticosterone) in human and rat plasma.

109

Determination of individual adrenocortical steroids in urine. E. Heftmann, D. F. Johnson, and Alma L. Hayden (*Section on Steroids, Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.*)

The method of Heftmann and Johnson [*Anal. Chem.* **26,** 519 (1954)] has been adapted to the determination of individual adrenocortical steroids in urine extracts. It is based on the automatic separation of the hormones on silicic acid columns with a stationary water phase by gradient elution with dichloromethane in petroleum ether. The extracts, prepared in the conventional manner after adjustment of urine to *p*H 1.0 or hydrolysis with glucuronidase are applied to the columns in dry form.

The chromatographic fractions are assayed by determination of ultraviolet

absorption and blue tetrazolium reduction and may be used for other qualitative or quantitative analysis. Owing to their load capacity, partition columns are relatively insensitive to impurities in the extracts and allow the determination of small amounts of one hormone in the presence of a large excess of others. The method is also useful on a preparative scale since the fractions are not contaminated by the chromatographic system. Excretion patterns are presented of normal men, pregnant and nonpregnant women, untreated patients with various metabolic diseases, and subjects under hormone therapy.

110

Determination of plasma corticosterone by isotope dilution. R. E. Peterson (*National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.*)

Corticosterone, like hydrocortisone, has been shown to be secreted by the human adrenal. Attempts to measure its concentration in the peripheral blood have, however, not been so successful as have determinations of the plasma hydrocortisone levels. This results from the fact that the plasma concentrations are so low and the methods that have been used have lacked specificity. We have developed a method of assay based on the principle of isotope dilution, using corticosterone-4-C^{14}. Paper chromatography has been used for the purification of the plasma extract, and a fluorometric assay for measuring the corticosterone. Extensive studies have been carried out to validate the specificity of this procedure. In normal subjects a range of 0.5 to 2.0 μg./100 ml., with a mean of 1.1, has been found. The levels have been found to increase under the influence of ACTH, and to decrease with suppression of the adrenal.

111

Urinary K/Na ratio and aldosterone activity. C. L. Fox, Jr., and S. E. Lasker (Flower-Fifth Ave. Hospital, New York Medical College, New York, N. Y.)

Aldosterone has been shown to induce marked sodium retention and relatively increased excretion of potassium. Thus the K/Na ratio of the urine may increase to extremely high levels (e.g. 100; normal K/Na = ±0.5–1.0). The urine of patients with edema associated with congestive heart failure, the nephrotic syndrome and cirrhosis of the liver, has been shown to contain abnormally large amounts of aldosterone (Luetscher, Neher, *et al.*). In postoperative surgical patients also increased quantities of aldosterone have been found in the urine (Llaurado). Following low sodium diets, the level of aldosterone in the urine has been found increased, and similar increases have also been observed in patients with certain forms of hypertension. In all these clinical situations, aldosterone per se, has been isolated and identified by combined chemical methods and bioassay. The urine samples of these patients exhibit high K/Na ratios. When therapy is directed at overcoming the *physiologic* activity of aldosterone—(a) by raising the sodium intake, (b) by administering diuretic agents, or (c) by steroid therapy which induces diuresis in the nephrotic syndrome—urine output gradually increases and the K/Na level is decreased by as much as a hundredfold. Data will be presented from a variety of clinical syndromes (thermal burns, anuria, surgical and medical patients) which suggest that the urinary K/Na ratio

parallels endogenous aldosterone activity: a high ratio indicates high levels of urinary aldosterone and that, when the physiologic electrolyte effects of aldosterone are suppressed, the K/Na level falls to low levels coincident with an increased output of urine.

112

The effects of adrenalectomy on serum lipid levels in the human. D. A. Sherber and M. Marcus (*Metabolic Researarch Laboratory, Fordham Hospital, Bronx, N. Y.*)

Following surgical ablation of the adrenal glands and gonads for breast cancer in male and female patients, the following changes were observed: A steady increase in serum cholesterol from normal levels to amounts exceeding 400 mg./100 ml.; phospholipids similarly increased but at a lesser pace resulting in an altered cholesterol/phospholipid ratio; total lipids increased as well as neutral fats, but at no time was there a visible lipemia. Several weeks after surgery were needed before the maximum effects were observed. These changes were independent of maintenance cortisone therapy as seen in the control group. The most striking change noted was in the freely extractable cholesterol of Macheboeuf. About 10 per cent of the total cholesterol is extractable by this method in the control groups which increased to about 50 per cent in the adrenalectomized human. Parallel changes were noted in a case of Addison's disease studied. All patients studied had normal renal, hepatic, cardiac, and respiratory functions as determined by laboratory tests. These patients also failed to demonstrate clinical or laboratory evidence of metastases.

113

Method for the isolation and determination of urinary aldosterone. W. Nowaczynski, E. Koiw and J. Genest (*Clinical Research Department, Hotel-Dieu Hospital, Montreal, Canada*) Abstract to follow.

114

Vitamin A absorption in mongolism. A. E. Sobel, M. Strazzulla, B. S. Sherman, B. Elkan, S. W. Morgenstern, N. Marius, and A. Meisel (*Departments of Biochemistry and Pediatrics, Jewish Hospital of Brooklyn, N. Y.*)

An investigation of the biochemical differences of blood of the mongoloid child as compared to the normal child in the same age group revealed the following deviations in mongolism: (1) vitamin A in oil absorption is lower. (2) serum gamma globulin is higher. (3) albumin is lower. (4) serum calcium is lower.

No significant deviations were found in the following components: potassium, sodium, bicarbonate, chloride, inorganic phosphate, total protein, A/G ratio, protein-bound iodine, total nitrogen, total iodine, total and free cholesterol, alkaline phosphatase, citric acid, sugar, urea, uric acid, bilirubin, α_1-globulin, α_2-globulin, β-globulin.

These studies will be expanded to explore the working hypothesis that the initial cause of mongolism may be related to an inadequate transfer from the mother to the fetus of vitamin A and possibly other fat-soluble vitamins.

115

The effect of various amounts of vitamin D on the incidence of hypocalcemia and hyperphosphatemia during the first week of life. J. B. Pincus and I. F. Gittleman (*Department of Pediatrics, The Jewish Hospital of Brooklyn, Brooklyn, N. Y.*)

Normal mature newborn infants were divided into the following groups: *Group A*, breast-fed infants; *Group B*, infants given processed milk formulas.

The group receiving breast milk was subdivided into Group 1 and Group 2. The infants in Group 1 received only breast milk. Group 2 received breast milk supplemented with 600 vitamin D units daily.

The group of infants receiving processed milk was subdivided in the following way: (1) infants receiving processed milk containing no vitamin D; (2) infants receiving processed milk containing 400 units of vitamin D per quart of diluted formula. The total intake of vitamin D for this group was approximately 400 units for the period studied; (3) infants receiving processed milk containing no vitamin D, but having 600 units of vitamin D added daily to their diet. The total intake of vitamin D for this group was approximately 3000 units; (4) infants receiving 3000 units of vitamin D daily in their milk formula. The total intake of vitamin D for this group was approximately 12,000 units for the period studied; (5) infants received during the period of study 45,000 units of vitamin D.

Blood samples were taken on the first day of life and on the day of discharge. Serum calcium and phosphorus were done on the first day of life and on the day of discharge.

Breast-fed infants receiving no vitamin D showed no rise in the serum phosphorus and no drop in the serum Ca by the end of the first week of life. Breast-fed infants receiving a supplement of vitamin of 600 units showed a rise of serum phosphorus and a tendency to a lowering of the serum calcium. In the infants receiving processed milk formulas with vitamin D those receiving 600 units of vitamin D daily showed the highest tendency toward an increase in the incidence of hypocalcemia. This increase in hypocalcemia reverses itself in the group receiving 3000 units of vitamin D, and when 45000 units of vitamin D was given there appeared a marked lowering in the incidence of hypocalcemia. The tendency toward hyperphosphatemia remained in all groups receiving processed milk formulas.

Vitamin D in high dosage has a parathormone effect. Since it has been postulated that newborn infants have a transient hypoparathyroidism in the neonatal period the effect of vitamin D observed in this study would tend to substantiate the hypothesis of transient hypoparathyroidism in the neonatal period.

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The effect of various kinds of feeding on the incidence of hypocalcemia and hyperphosphatemia during the first week of life. J. B. Pincus and I. F. Gittleman (*Department of Pediatrics, The Jewish Hospital of Brooklyn, Brooklyn, N. Y.*)

Groups of infants were fed breast milk, diluted fresh cow's milk, diluted evaporated and powdered milks. Serum calcium and phosphorus levels were determined on the first day of life and on the day of discharge from the hospital.

Infants who were breast fed showed no alteration in the level of serum calcium or serum phosphorus when these levels were normal at birth. When the serum calcium was low at birth there was a tendency for the level to rise. The incidence of hypocalcemia was about 4 per cent in children receiving diluted fresh cow's milk. The incidence of hypocalcemia in the group receiving evaporated milk was 17 per cent and in the group on powdered milk was 15 per cent.

Breast milk appears to prevent neonatal hypocalcemia. Fresh cow's milk causes the serum phosphorus to rise but its hypocalcemic effect is slight while processed milks affect the highest rise in serum phosphorus and cause an increased incidence in the hypocalcemia in newborns during the first week of life.

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Vitamin B_{12} in serum and urine in pregnancy. H. Baker, Ruth Erdberg, and H. Sobotka (*Departments of Chemistry and Obstetrics, The Mount Sinai Hospital, New York, N. Y.*)

A survey of the serum and urine of pregnant women shows that during the first trimester of pregnancy the urine values of cyanocobalamin are similar to those in nonpregnant subjects. The serum values indicate the same trend. During the second and third trimester the urine values remain the same; however, the serum values decrease to levels comparable to those met in pernicious anemia. Correlation of these findings with the vitamin B_{12} levels in fetal blood will be discussed. The assays were carried out in parallel with *Euglena gracilis* and *Ochromonas malhamensis.*

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Studies of the diacetyl reaction for determination of urea. W. H. Marsh, B. Fingerhut, and E. Kirsch (*State University of New York, Department of Pathology, School of Medicine and Kings County Hospital, Brooklyn, N. Y.*) Abstract to follow.

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Techniques for pernicious anemia diagnosis with radiocyanocobalamin. D. L. Tabern and R. H. Storey (*Department of Radio-Pharmaceuticals, Abbott Laboratories, North Chicago, Illinois*) Abstract to follow.

SCIENTIFIC EXHIBITS

Micro modification of the Van Slyke-Neill manometric apparatus. D. D. Van Slyke and J. Plazin (*Brookhaven National Laboratory, Upton, N. Y.*)

A micro chamber of 10 ml. capacity replaces the 50-ml. chamber of the standard Van Slyke-Neill apparatus. The micro and standard chambers are interchangeable without alteration of the rest of the standard apparatus. The micro chamber permits measurement of pressures with gas volume at 0.1 ml. with accuracy similar to that obtained with the standard 50-ml. chamber with the gas at 2.0 ml. volume. For analyses of blood gases, samples of 50 or 100 cubic millimeters of blood or plasma are measured from a micro form of Guest's pipet provided with a stainless-steel needle tip. The various other analyses described for the standard apparatus can be done with micro samples in the smaller chamber.

Color reactions of amino acids and their determination in protein hydrolysates with circular paper chromatography. I. Oreskes and A. Saifer (*Department of Physical Chemistry, Isaac Albert Research Institute, Brooklyn, N. Y.*)

Circular paper chromatography using two solvent systems and two coloring reagents was employed for the qualitative identification of amino acids in protein hydrolysates. Solvents used were phenol-0.1% NH_3 and butanol-acetic acid-water (4:1:5). Two coloring reagents, ninhydrin and isatin, were employed. Because of its selectivity isatin is useful in identifying certain amino acids even when these were not completely resolved. The method has the advantages of simplicity, speed, and compactness. More accurate R_f's are obtained and several coloring reagents may be used on one chromatogram.

The technic has also been applied to the quantitative analysis of insulin. For this work the arc rather than the complete circle method was used. This enables one to run both unknown and standards on the same filter paper. The colored arcs were eluted and read in a spectrophotometer. The values obtained compared favorably with previous analyses of this protein.

Color reactions of 51 amino acids with ninhydrin, isatin, and alloxan have been studied and their sensitivity limits determined. The circular method was employed to determine R_f values with phenol and butanol solvents. Isatin gives useful reactions with about 35 amino acids. This semi-specificity makes it very useful as a "differential" color reagent. Alloxan reacts with virtually all amino acids and in some cases the reaction is more sensitive than with ninhydrin. In addition the colors obtained on paper with alloxan are more stable and uniform than those obtained with ninhydrin. This suggests its potential usefulness as a general reagent for amino acids.

A complete toxicological service in a general hospital. S. Nobel (*Monmouth Memorial Hospital, Long Branch, N. J.*)

In recent years, the clinical chemist has been confronted with an increasing number and variety of toxicologic problems. To a large extent this is due to the advances in chemical technology which have introduced a wide range of noxious chemicals into the home area, and the carelessness of parents in storing these materials safely out of their children's reach. Furthermore, the recent rise in the number of poison control centers established throughout the country by pediatric and citizen's groups is indicative of public awareness of the clinical chemist in either centers of this type or in the individual hospital.

The purpose of this exhibit is to present useful information and suggestions that have been of practical value in dealing with toxicologic cases. Our views are based on background experience gained in the Laboratory of Dr. A. O. Gettler, Chief Toxicologist to the Medical Examiner of the City of New York, as well as the incorporation of a complete toxicologic service in the laboratory of a 300-bed general hospital.

The role of the clinical chemist in handling a toxicologic problem as demonstrated in the exhibit has been divided into the following five critical areas:

1. Selection of literature to cover the clinical picture and therapy, chemical methods, and information on product composition.
2. Orientation of the emergency room to work in close conjunction with the laboratory.
3. General rules of procedure.
4. Outline of several specific methods with illustrative case histories.
5. Participation in public health education programs.

Instrumental solutions to some common laboratory problems. R. L. Dryer and J. I. Routh (*Clinical Biochemistry Laboratory, College of Medicine, State University of Iowa, Iowa City, Iowa*)

Many common problems in the clinical chemistry laboratory can be solved by simple apparatus or implements not ordinarily available on the market. Development of feasible ideas is often limited only by the irritation aroused by the problem itself.

We will demonstrate several devices developed in our laboratory as a result of our dissatisfaction with existing technics. Among these will be a device for isothermal distillation of ammonia designed originally for BUN determinations on 0.05 ml. blood in 30 minutes, but useful also for any system wherein a gaseous component is generated. A second item is a universal cuvet which may be made an integral part of a spectrophotometer, thereby insuring constancy of optical conditions in any series of measurements, and ending concern for axial alignment of the cell. Among the remaining parts of the exhibit will be a semimicro liquid-liquid extractor which can be used with liquids either heavier or lighter than water with rapid stripping of desired solutes and minimal foaming or emulsifying tendency.

Operational details will be made available to those who are interested in any of the items exhibited.

Glucagon and the anti-insulin action of cortisone. B. W. Volk, and S. S. Lazarus (*Jewish Chronic Disease Hospital, Brooklyn, N. Y.*)

Intravenously administered regular insulin or subcutaneously administered regular or protamine-zinc insulin cause a marked initial hyperglycemia in cortisone pretreated rabbits. The height of the hyperglycemia depends on the amount of glucagon present in the insulin preparation. Similarly, glucagon alone or combined with various amounts of glucagon-free insulin cause a marked initial hyperglycemia whose height is proportional to the glucagon dosage. Increasing the amount of insulin in the mixture does not result in a proportional diminution in the hyperglycemic action of the glucagon. The paradoxical hyperglycemic action of commercial insulin and the augmented effectiveness of glucagon are not due to the cortisone-induced diabetic state per se, since they are not observed in alloxan-diabetic animals.

These findings suggest that the insulin antagonistic action of cortisone is due, at least in part, to potentiation of the action of glucagon. Thus, it may be advantageous to utilize glucagon-free insulin preparations in the treatment of various conditions in which hyperadrenocorticalism is a factor.

Micro methods in the clinical laboratory. S. Natelson (*Rockford Memorial Hospital, Rockford, Ill.*)

By means of color charts and equipment a system is described for practical application to the clinical laboratory of ultramicro technics. The sample size for most procedures is of the order of 0.01 to 0.05 ml.

Procedures are described for the usual constituents found in blood such as sugar, urea, protein, electrolytes, calcium, bilirubin, etc.

A rapid system of detection of toxic substances in the blood is described including barbiturate, salicylate, lead, arsenic, mercury, cyanide, methyl and ethyl alcohol, carbon monoxide, and morphine.

Demonstrations are held for determination of *p*H, CO_2, and ultramicro titrations.

Percutaneous and oral absorption of vitamin A. A. E. Sobel and B. S. Sherman (*Department of Biochemistry, Jewish Hospital of Brooklyn, N. Y.*); Histological studies by Jerome P. Parnell (*Department of Anatomy, State University of New York, College of Medicine at New York City*)

Studies on vitamin A-deficient and normal rats show that the percutaneous absorption of vitamin A is about $\frac{1}{5}$ to $\frac{1}{10}$ as efficient as oral absorption. The normal skin is more efficient in the percutaneous absorption of vitamin A than the A-deficient skin.

Histologic studies show restoration of skin structure at the site of application but not at untreated sites. There is a time lag between the development of A-deficiency, as shown by xerophthalmia, body weight loss, and skin symptoms of deficiency.

The nature of the medium affects the transfer of vitamin A across the skin, as will be shown by various charts.

Vitamin E in combination with vitamin A produced increased storage of vitamin A when topically applied or orally administered.

Data will be presented on the influence of dosage, either orally administered or topically applied, on the liver storage of vitamin A in normal and in A-deficient animals.

Spectrophotofluorometry: A new tool for analysis at the sub-microgram level. D. E. Duggan and S. Udenfriend (*Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Bethesda, Md.*)

The exhibit consists of a demonstration of the operation of at least one of our three instruments, and an exhibit board illustrating the fundamental concepts of fluorimetry, comparing spectrophotofluorometry to conventional fluorimetry, and showing reproductions of graphic data illustrating typical applications to both qualitative and quantitative analysis.

For description see abstract, Scientific Session 5.

Eight- to twenty-four-hour Frank-Berman pregnancy test. Rose L. Berman (*Berman Clinical Laboratory, New York, N. Y.*)

The Frank-Berman pregnancy test, first described in 1941, is the first successful pregnancy test using rats with results in 8 and 24 hours. The test is a very simple

one, utilizing two immature female rats, weight 50 Gm. (weight range, 45–60 Gm.).

Urine specimen to be tested is centrifuged and clear supernatant urine which contains the chorionic hormone is used. Rats are given two subcutaneous injections, 5.0 ml. at each injection, the first injection administered in the morning, the second injection in the afternoon, four hours being minimum time interval between injections.

The following morning, 16–24 hours after the first injection, rats are sacrificed with illuminating gas. Ovaries are dissected out via the abdominal route and are read grossly with naked eye. A negative ovary is small, pale and creamy in color or slightly pinkish, the surface of the ovary showing minute colorless follicles. A positive ovary is usually, but not always, enlarged and is entirely reddened or may have many distinct red spots on it. The test, if performed as described and all precautions observed, is 100 per cent accurate.

The Frank-Berman pregnancy test will also yield results in 8 hours. However, the value of the 8-hour test is limited since only positive results are conclusive. A negative test cannot be considered a true negative unless confirmed by the 16–24 hour test.

The Frank-Berman pregnancy test is used to diagnose pregnancy, the presence of retained fragments of placenta or chorionic tissue, hydatidiform mole, and chorionepithelioma in the male as well as the female.

Principles and results of paper electrophoresis of proteins. Hub. Peeters (*Brugge, Belgium*)

This exhibit gives a survey of the factors influencing migration of proteins in paper electrophoresis and their subsequent evaluation. Each physical and physicochemical factor is considered separately and its variations demonstrated by means of actual paper strips and diagrams. Both uni- and bidimensional technics are considered.

Migration is influenced by the structure of the apparatus and by physical forces involved such as temperature, hydrodynamics, and evaporation. The characteristics of the substrate and the buffer solution are summarized. The electrical field causes both ionophoresis of the buffersalts and electrophoresis of the larger molecules. The interaction of these forces is demonstrated.

International Biochemical Trial 1954

I. D. P. Wootton

ANALYTICAL TRIALS have been carried out in several countries. Generally, samples of a single specimen were circulated to a number of chemical laboratories for the analysis of certain constituents (for references see *Lancet 264:* 476, 1953). Inspection of the results has usually shown an unexpectedly large variation between laboratories, indicating a most unsatisfactory level of precision in clinical analyses as generally practiced.

In 1953, the Committee of the International Federation of Clinical Chemistry decided to conduct such a trial on an international scale. The I.F.C.C. was fortunate in receiving financial support from the International Union of Pure and Applied Chemistry. The Committee is also indebted to the Wellcome Research Institute, London, which prepared and donated the samples used. Organization was complete and the samples ready by January, 1954.

TEST MATERIAL

The material used was horse serum, measured accurately into vials and freeze-dried. Two separate samples were analyzed by each laboratory, one being derived from the original serum (Sample 37A) and the other from an accurately made dilution of it (Sample 37B). This was done so that the ratio between the two results could be examined as well as the absolute value obtained. The Wellcome Institute arranged that the liquid in 37B should be precisely 70% as concentrated as 37A, but in reconstitution an equal amount of water was added to each sample and calculation, allowing for the specific volume of the solids, indicates that the ratio 37B/37A should be about 72%.

Secretary, International Federation of Clinical Chemistry, Post-Graduate Medical School, London, England.

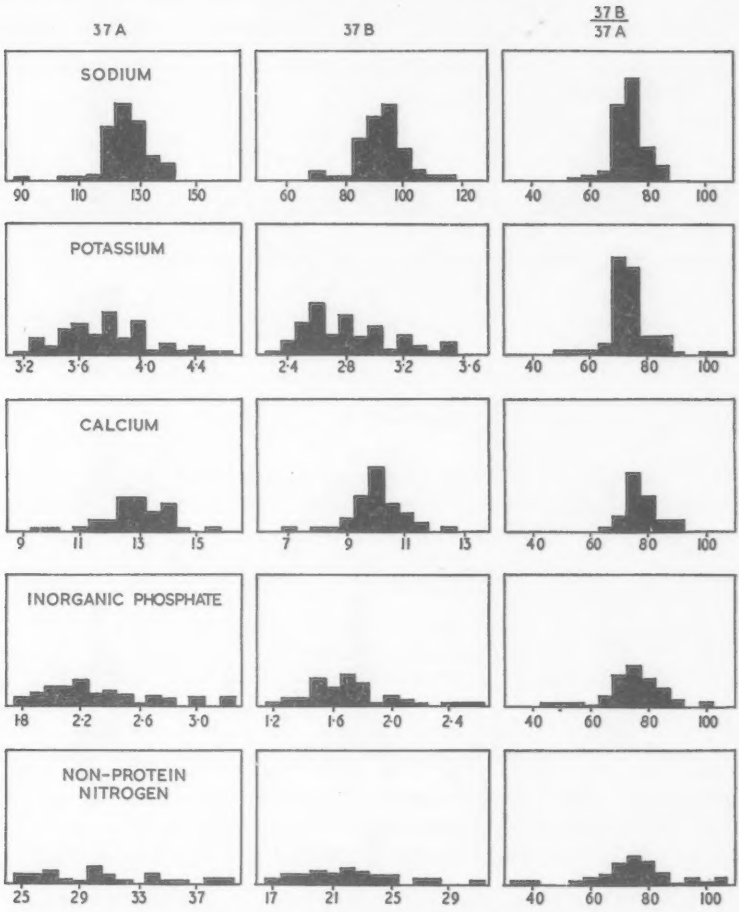

Fig. 1. Distribution of results received, together with the ratio 37B/37A. Units of results are given in Table 1.

DISTRIBUTION OF SAMPLES

Distribution within the countries concerned was arranged by the I.F.C.C. national representatives or their nominees. The numbers of samples distributed were as follows: France 30 (8); Italy 10 (7); Netherlands 40 (33); South Africa 1 (1); Scandinavia 25 (13); United States 30 (20); United Kingdom 52 (49); Yugoslavia 2 (2). The figures in parentheses are the number of reports received.

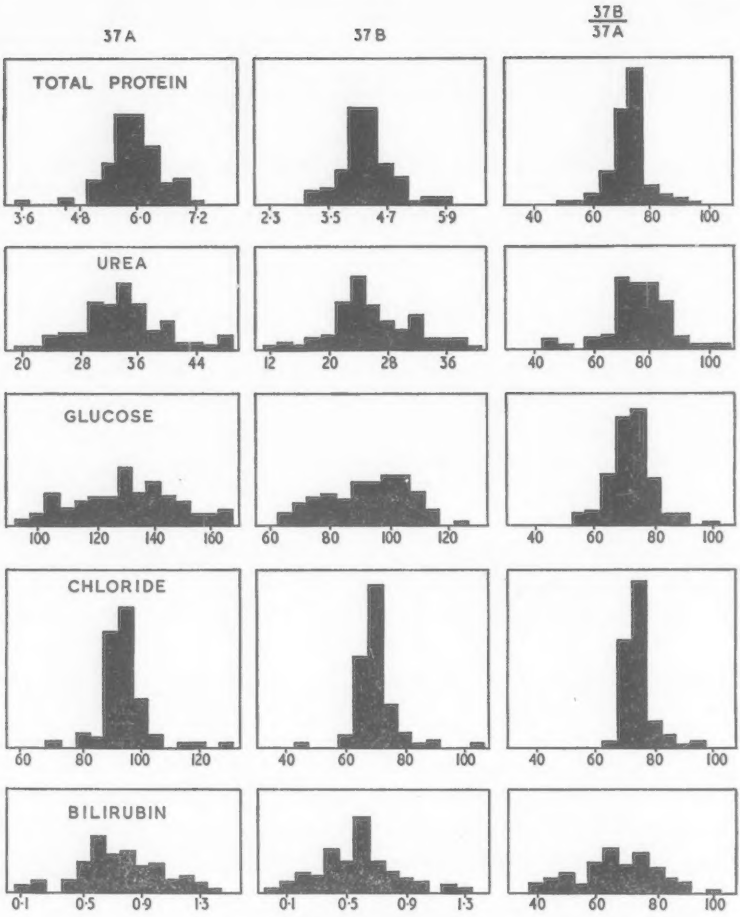

Fig. 1. Continued

RESULTS

In Fig. 1 the distribution of the reported results is shown. These are the combined results of all the countries concerned. The values are given in the units mentioned in Table 1, which also shows how many determinations were received, and how many are outside (above or below) the limits of the diagram. The ratio 37B/37A is plotted as a percentage, using the true value as 72%. Individual histograms plotted for

Table 1. Number of Determinations Received and the Number Falling Outside the Limits of the Diagrams

Constituent	*Units in Fig. 1*	*Total no. of results*	*No. outside the limits of diagram*		
			37A	*37B*	*Ratio*
Sodium	mEq./L.	125	2	2	0
Potassium	mEq./L.	103	8	9	0
Calcium	mg./100 ml.	129	1	0	0
Inorganic phosphate	mg.P/100 ml.	121	5	12	5
Nonprotein nitrogen	mg./100 ml.	102	9	5	0
Total protein	Gm./100 ml.	93	2	1	2
Urea	mg./100 ml.	99	5	5	5
Glucose	mg./100 ml.	60	5	4	0
Chloride	mEq./L.	65	0	4	3
Bilirubin	mg./100 ml.	40	3	0	11

each country indicated no marked differences in precision between one country and another.

Table 2 shows the mean values arranged by countries. These have been calculated to demonstrate any significant differences between countries in the general level of results. The most striking difference is in glucose results between the United States and the remainder. At first sight this might be taken to be a reflection of the methods used, by postulating that the United States generally reported "true sugar" while most of the remainder reported "total reducing substances." Reference to the report forms, however, does not support this view and there seems to be no ready explanation.

CONCLUSIONS

There are two conclusions to be drawn from this trial. In the first place, this problem is universal and no country taking part maintains an acceptable standard of laboratory precision. Secondly, inspection of Fig. 1 shows that the ratio 37B/37A is significantly more constant for most constituents than are the corresponding absolute values. This implies that laboratories can compare two solutions more accurately than they can determine the absolute value of a constituent. It follows that estimations should be made whenever possible by comparing an unknown with a standard solution, a measure that would immediately eliminate one source of error.

PLAN FOR REFERENCE STANDARDS

As a result of the publication of trial results of this kind, there is now a much more general appreciation of the problem. One suggestion to im-

Table 2. Samples Analyzed and mean Values of Estimations Arranged by Countries
(Units as in Table 1)

	Sodium		*Potassium*		*Calcium*		*Inorganic phosphate*		*Nonprotein nitrogen*		*Total protein*		*Urea*		*Glucose*		*Chloride*		*Bilirubin*	
	37A	*37B*	*37A*	*37B*	*37A*	*37B*	*37A*	*37B*	*37A*	*37B*	*37A*	*37B*	*37A*	*37B*	*37A*	*37B*	*37A*	*37B*	*37A*	*37B*
Netherlands	126	93	3.75	2.73	12.9	9.9	2.2	1.8	30	22	5.7	4.3	32.0	24.7	136	100	94	68	.71	.49
Scandinavia	129	93	3.71	2.82	12.7	9.9	2.7	2.3	29	21	5.6	3.9	28.0	20.1	133	97	92	70	.97	.70
United Kingdom	124	92	3.98	2.99	13.1	10.1	2.4	2.0	33	25	6.0	4.3	34.2	26.8	133	98	94	71	.83	.56
United States	126	90	3.6	2.58	12.9	9.8	2.3	1.8	26	20	6.0	4.4	..	..	103	72	95	73	.77	.62
Others	138	105	3.95	2.70	..	..	..	2.6	..	..	5.6	4.6	32.2	24.7	126	88	97	73	.84	.55
Grand Mean	126	92	3.81	2.81	12.9	10.0	2.4	2.0	30	22	5.9	4.3	32.9	25.5	129	93	94	71	.80	.55

prove matters is now being tried out. This involves the preparation of samples similar to those used in this trial, but calibrated for the content of various constituents by suitable reference laboratories. Individuals can then compare their own results with those of the reference laboratories.

Several countries are considering or have already started such projects, which promise to be of very great value especially to smaller and more isolated laboratories. It is also hoped that the reference laboratories in each country will be able to test the samples of each other's national plans in order to detect differences in level between countries such as indicated by the glucose results of Table 2.

HILL ELECTED AACC PRESIDENT

Robert M. Hill, Professor of Biochemistry at the University of Colorado Medical School, was elected President of the American Association of Clinical Chemists to serve from July 1, 1956, to June 30, 1957. Professor Hill was born in Carthage, Illinois, and received his graduate training at the University of Illinois where he was awarded a Ph.D. in Biochemistry in 1923. He has served at the University of Colorado since 1925, and in his present position since 1944. He was on the Executive Committee of the Association for several years and was vice-president for the 1955–56 term.

The other officers who will serve for the same period include *Joseph I. Routh*, State University of Iowa, Iowa City, vice-president; *Max M. Friedman*, Lebanon Hospital, New York, national secretary; and *Louis B. Dotti*, St. Luke's Hospital, New York, national treasurer.

The members of the National Executive Committee for 1956–57 consist of *Emmett B. Carmichael*, University of Alabama Medical College, Birmingham; *William E. Cornatzer*, University of North Dakota Medical School, Grand Forks; *George R. Kingsley*, Veterans Administration Center, Los Angeles, Calif.; *Martin I. Rubin*, Georgetown University Medical School, Washington, D. C.; and *Otto Schales*, Alton Ochsner Medical Foundation, New Orleans, La.

The officers and members of the Executive Committee compose the governing body of the Association. They were elected by the membership from a slate recommended by the Nomination Committee. The ballots were tabulated and the election certified by the official tellers, Albert Hanock and Andre C. Kibrick.

NOMINATING COMMITTEE

A Nominating Committee was elected by the membership. This committee is authorized to propose a slate of officers for the 1957–58 National Executive Committee.

The Nominating Committee will consist of *John G. Reinhold*, University of Pennsylvania, Philadelphia, to serve as chairman; *Monroe E. Freeman*, Medical Service Corps, Washington, D. C.; *Joseph H. Gast*, Baylor University, Houston, Texas; *Samuel Natelson*, Rockford Memorial Hospital, Rockford, Ill.; *Miriam Reiner*, District of Columbia General Hospital, Washington, D. C.; *Albert E. Sobel*, Jewish Hospital, Brooklyn, N. Y.; and *Harry Sobotka*, Mount Sinai Hospital, New York. *Oliver H. Gaebler*, Henry Ford Hospital, Detroit, Mich., was elected as alternate.

CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

Information for Authors

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The original (on bond paper) and first carbon of the manuscript should be submitted, triple spaced with ample margins all around. The author's last name should appear on each page. Separate pages should be used for title page (with author's name and affiliations), references, footnotes (when unavoidable), illustration legends, tables, and other inserts.

References and the design of tabular matter should follow exactly the form used in current issues of this journal. Journal abbreviations should conform to the style of *Chemical Abstracts*. The accuracy of the references and the provision of adequate bibliographic data are the responsibility of the author.

A reasonable allowance is made for black and white line cuts and tabular composition. If this allowance is exceeded, or if halftones or color plates are required, special arrangements will have to be made with the Chairman of the Editorial Board.

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**Saifer, A., and Deutscher, C. "A Study of Bovine Serum Ultrafiltrate as a General Standard in Clinical Analysis". Clinical Chem., January, 1956.*

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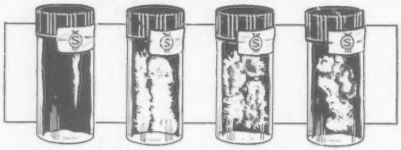

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